"ARTIFICIAL UPWELLING"

PROGRESS 1976-1977

(PRELIMINARY)

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C O N T E N T S

INTRODUCTION

This preliminary progress report describes what was accomplished since July 1, 1976, under Sea Grant support, on the "Artificial Upwelling" mariculture project.

—During this year we have started a pilot demonstration plant, operated on the basis of the best information we had collected previously.

—We subjected this pilot operation to a set of observations to verify the validity of our method of operation.

—A series of developmental research studies were undertaken, to increase our understanding, or improve the productivity, of the processes involved.

—The "inputs" on which the pilot plant operation is based are summarized in a technical description.

—The procedures which were applied in all phases of our effort have been identified.

—The data collected, both as a result of observations and of research studies, have been subjected to an initial analysis.

—The final output of this program is the substantiation of our aquaculture budget generator program, which now provides us with guidance as to the economically critical aspects of our project, and will ultimately provide us with the rationale for further development.

1. TECHNICAL DESCRIPTION

The planning and operation of our pilot demonstration plant and the analysis of the results obtained require an adequate set of quantitative relationships between the mariculture's outputs, its inputs, and its environment. These relationships are derived from experimental results and from our understanding of the mechanisms involved, which also guides us in interpreting these results.

The initial technical description, undertaken prior to the generation of our plan of work for 1976-77, identified the operational parameters then in use, such as turnover rates, shellfish feeding rates, etc., and the measured phytoplankton and shellfish yields. Such a description is adequate within the range experimentally verified, and as long as all reliable observations confirm the fundamental concepts.

No revision of the initial technical description has been completed. Our present pilot operation, output prediction and economic evaluation are still based on the initial description, a copy of which is included as Appendix A.

Observations performed on our pilot plant operation are not in agreement with predictions based on smaller-scale quantitative experiments described in our "constant weight" study (Appendix B). The reason for discrepancies, and the extent to which the description has to be amended, are being examined and are probably related to the scale-up of the shellfish-growing system or to the switch to a unialgal diet

adopted in the pilot plant operation. In the interim, the set of data on which the initial technical description is based is representative of a normal operation and more recent observations are considered anomalous.

2. DESCRIPTION OF PROCEDURES

2.1 Pilot Plant Operation

2.1.1 Phytoplankton

The deep water that we are pumping on St. Croix from a depth of 870 m is an excellent medium in which to grow photosynthetic plants, but it contains very few of them because they are not able to multiply in the absence of light at that depth. The purpose of the algal mass culture system of the mariculture plant is to provide dense "starter" cultures (for the inoculation of deep water) of the desired kinds of algae that are known to satisfy the requirements of filter-feeding shellfish. This is accomplished by producing axenic algal cultures in the laboratory in highly enriched sea-water medium under controlled environmental conditions, and using these cultures to inoculate still larger cultures on the beach, where temperature, light intensity, and other factors cannot be easily manipulated. The phytoplankter used is Chaetoceros curvisetus (clone STX-167).

The algal cultures are produced on a production-line basis daily by transfer of small cultures at intervals into larger growth vessels containing new media. The

last stage in the algal culture system is a large-volume culture in 13,000-gallon concrete pools, from which the algae are pumped continuously to the shellfish-rearing tanks. The laboratory and beach algal culture methods are further detailed. A schematic diagram of the flow through the algal culture system is given in Figure 1.

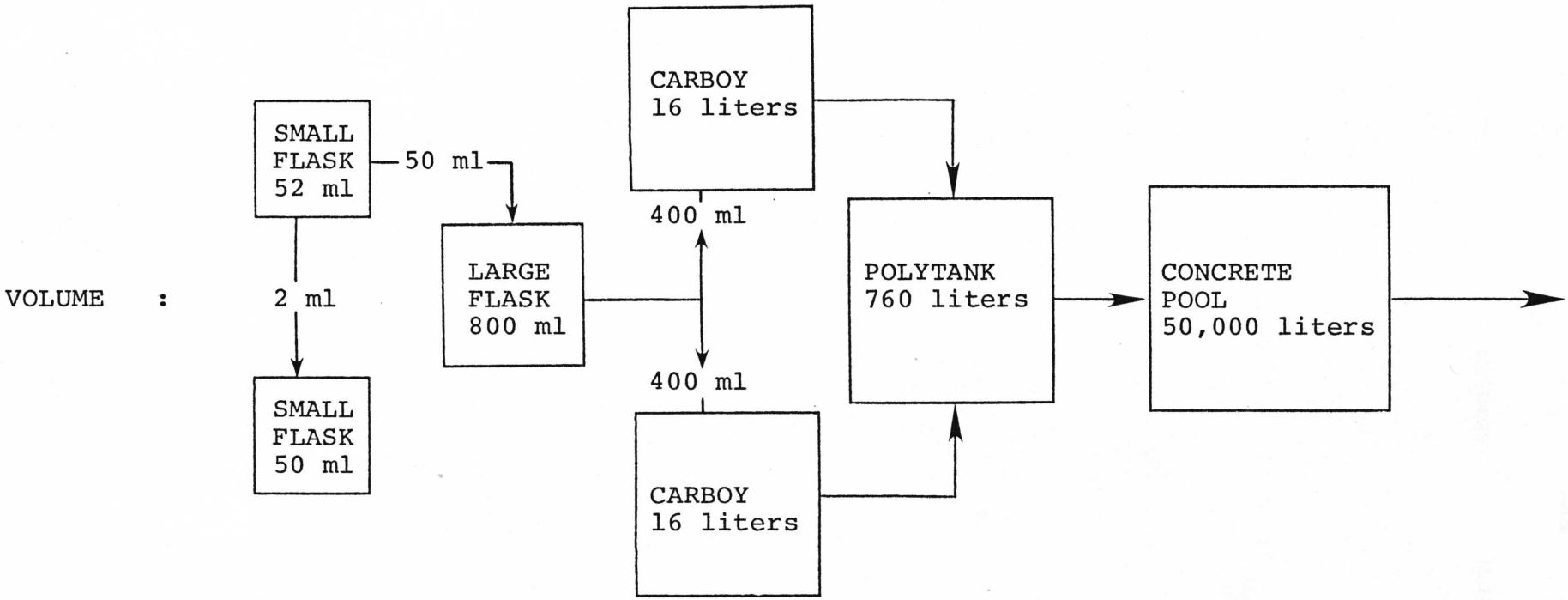
2.1.1.1 Laboratory Cultures

Inocula for the outdoor pools are reared as axenic laboratory cultures (16-liter carboys) which are used to inoculate the 200-gallon (760-liter) unialgal polytank (PT) cultures which, in turn, are used to inoculate the 13,000-gallon pools. Preparation of the laboratory cultures includes growing the the diatom in a 50-ml small flask (SF) for two days and using it to inoculate an 800-ml large flask (LF). The LF, after two days growth, is used as inoculum for two 16-liter carboys which are incubated in the laboratory for two days prior to being used as inoculum for a polytank. The SF is incubated in a 12-hr light/12-hr dark cycle; the LFs and carboys are incubated under continuous illumination. Illumination is provided by fluorescent light bulbs; temperature is normal room temperature (ca 25°C). The medium used in laboratory cultures is Guillard's medium F/2, except that silica is omitted from the SFs and LFs.

2.1.1.2 Beach Cultures

The medium used in the outdoor polytanks is F/4 (except for the F-level silica). The polytanks are inoculated with two 16-liter carboy cultures from the laboratory. Aeration is provided by a

Figure 1. Schematic diagram of the flow through the algal culture system.


SMALL FLASK 52 ml
50 ml
CARBOY 16 liters
400 ml
LARGE FLASK 800 ml
POLYTANK 760 liters
CONCRETE POOL 50,000 liters
VOLUME :
2 ml
SMALL FLASK 50 ml
400 ml
CARBOY 16 liters
STERILITY:
YES
NO
LOCATION :
INDOOR (LABORATORY)
OUTDOOR (BEACH)

polyvinylchloride (PVC) manifold which rims the bottom of the polytank. The PT is prepared for inoculation by scrubbing with a solution of household bleach (sodium hypochlorite) and a swimming-pool chloride (sodium dichloro-isocyanurate), filling with deep water and adding the enrichment nutrients. Carbon dioxide is metered into the PT cultures when the pH goes above 8.7.

The two 13,000-gallon (50,000-liter) concrete pools of approximately 1-meter depth, are provided with a continuous flow of deep water at a turnover or dilution rate of 1.15 pool volumes per day. Culture is pumped out continuously, using submersible pumps. Pool culture produced in excess of the needs of the pilot plant requirement is used for maintenance of brood stock and other animals, or overflows to waste. The pools are scrubbed (as for the PTs) and reinoculated on a 28-day cycle, so that a different pool is inoculated every two weeks. Following scrubbing of the pool and airlines, and flushing of the lines which distribute the culture to the pilot plant and other animal tanks, the pool is filled to half-volume, inoculated with a PT culture and allowed to sit overnight. The following day, the pool is filled to 13,000 gallons and is activated by starting continuous flow of deep water in and culture out. One inoculum PT culture is produced every week so that there is a backup PT culture available in the event that a pool culture "collapses" below a useful level (ca 10^4 cells/ml). The decision to scrub a pool on a day other than the

scheduled day is made by the beach technician, based on visual estimation of pool density. A 0.3-m (1-ft) diameter disk, having alternating black and white quadrants, is located on the bottom of the pool to aid in estimating the cell density of the pools.

If a pool collapses on a day other than when a PT culture is available, the pool is deactivated (flow in and out stopped) and placed under observation for possible recovery.

If a pool fails after one week or less, it is reinoculated and run for three weeks.

If a pool fails after two weeks, the pool is reinoculated and run for six weeks, the life of the other pool is extended an additional week before scrubbing, and then is run for three weeks before the next scrubbing, which brings both pools back to the original schedule.

2.1.2 Shellfish

2.1.2.1 Operation

The shellfish pilot plant, located on the beach west of the phytoplankton pools and perpendicular to the northeast corner of the hatchery building, consists of two parallel rows of six rectangular tanks (approx. 86 x 62 x 22 cm), which incorporate an airlift recirculation system (Figs. 2, 3). Tanks 1, 3, 5, 7, 9 and 11 receive flow from Pool 2, while Tanks 2, 4, 6, 8, 10 and 12 receive flow from Pool 1. All tanks can receive from one pool when only one is on-line.

Figure 2. Diagram of the shellfish pilot demonstration plant, consisting of two parallel rows of six rectangular tanks (approx. 86 x 62 x 22 cm) and incorporating an airlift recirculation system.

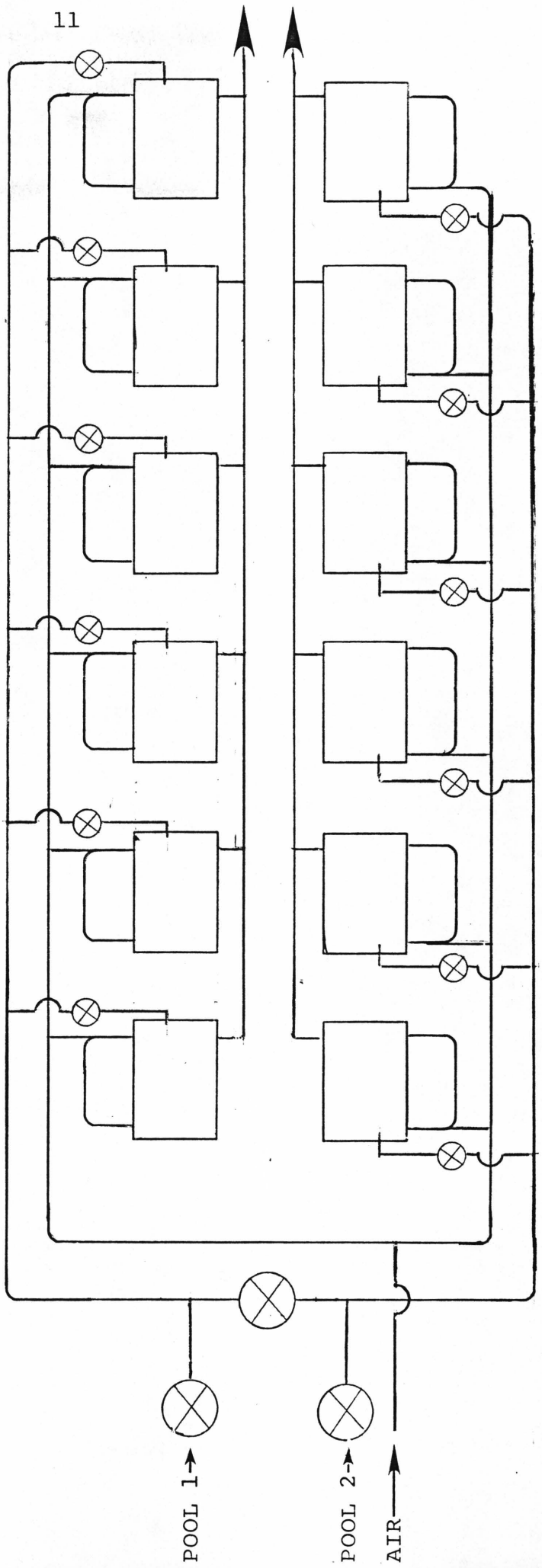
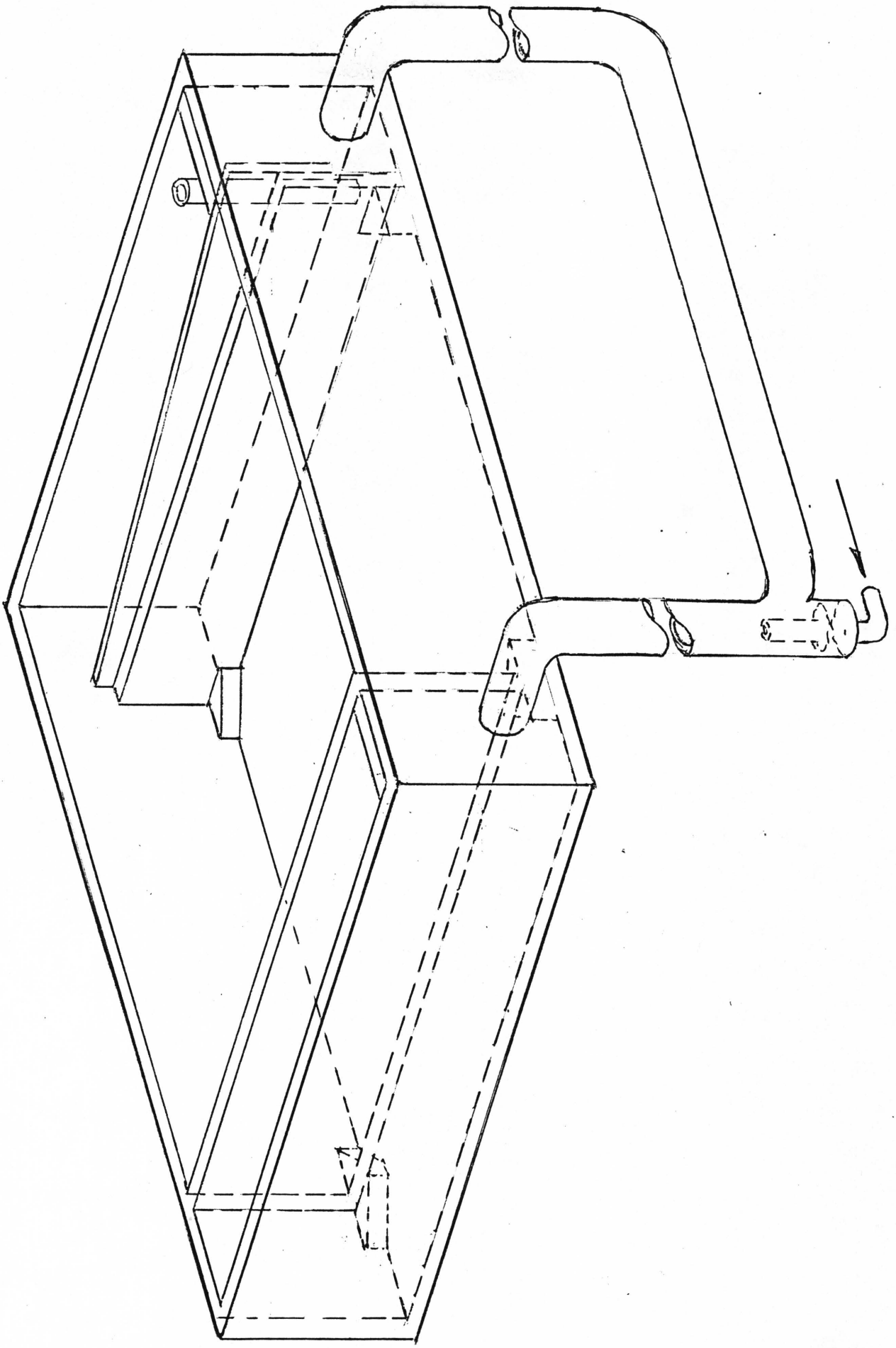

POOL 1
POOL 2
AIR

Figure 3. Another view of the shellfish pilot plant consisting of six covered rectangular tanks.

TO DRAIN
POOL 2
POOL 1

Figure 4. Detail of an individual shellfish tank.



The tanks are constructed of 5/8" exterior plywood with the main shellfish compartment separated from the inflow and outflow chambers by 3/4" plywood partitions (Fig. 4). The tanks are made watertight by trim-fitting corners and applying exterior spackling compound to fill the seams, then covered with polyester resin. The tanks are painted with a non-toxic, non-yellowing, white enamel paint. Covers for the tanks are made from corrugated aluminum roofing material.

Low-pressure air, supplied by a Siemens compressor (Model #2CH4-001-1U), is injected through capillary tubing at the base of the left-vertical section of the external recirculation system (Fig. 4), creating a recirculation flow through the tank. Pool culture entering from the left side of the tank flows under the inflow partition, up through a Nestier tray via the main compartment containing shellfish, and over the effluent chamber partition. A portion of the flow then exits via the overflow standpipe and the remainder is recirculated back through the tank.

Tank capacity is 113 liters and tank turnover time is 17 min at a flow of 110 ml/sec. The pilot plant requires the total flow from both pools when operating at full capacity.

Tapes japonica spat (approx. 10,000 clams) are introduced into the pilot plant 56 days after spawning at an expected shell length of 4.3 mm (0.1 g). The clams are placed in a 1/16" mesh bag in one-fourth of the Nestier tray and a weighted wood plate is used to cover the

remaining 3/4ths of the tray. This directs the flow of pool culture up through the clams when the entire tray is not being used. As the clams increase in size, a larger open-mesh Vexar screening replaces the mesh bag.

The subsequent feeding plan is based on a projected growth, from this size on, as derived from a 36-day feeding experiment (Roels et al., 1977; Appendix B) in November-December 1975, which used animals averaging 12.7 mm in length at the start.

On Day 70 the wood partition covering 3/4ths of the Nestier tray is removed and the shellfish are spread out on a tray liner over the entire tray. At Day 126, the shellfish are divided equally into quarters and spread out into four trays of the pilot plant. One batch occupies Tanks 1-4, the second occupies Tanks 5-8, and the third occupies Tanks 9-12. Tray liners are removed when the clams are large enough to be retained in the Nestier tray.

The expected growth of a population of 10,000 clams is shown in Figure 5. The predicted feeding flow required is shown in Figure 6. Actually, the flow is adjusted stepwise, as shown in Table 2.

After 140 days, three batches of 10,000 clams are expected to need a flow rate which exceeds our present pool capacity. Accordingly, after that date the shellfish population is reduced every 28 days, by culling the excess over a predetermined weight. The weight to which each population (four trays) has to be reduced each time, and the amount expected to be culled is shown in Table 3.

Figure 5. The expected growth of a population of 10,000 clams (Tapes japonica).

K+E 20 X 20 TO THE INCH • 7 X 10 INCHES
KEUFFEL & ESSER CO. MADE IN U.S.A.

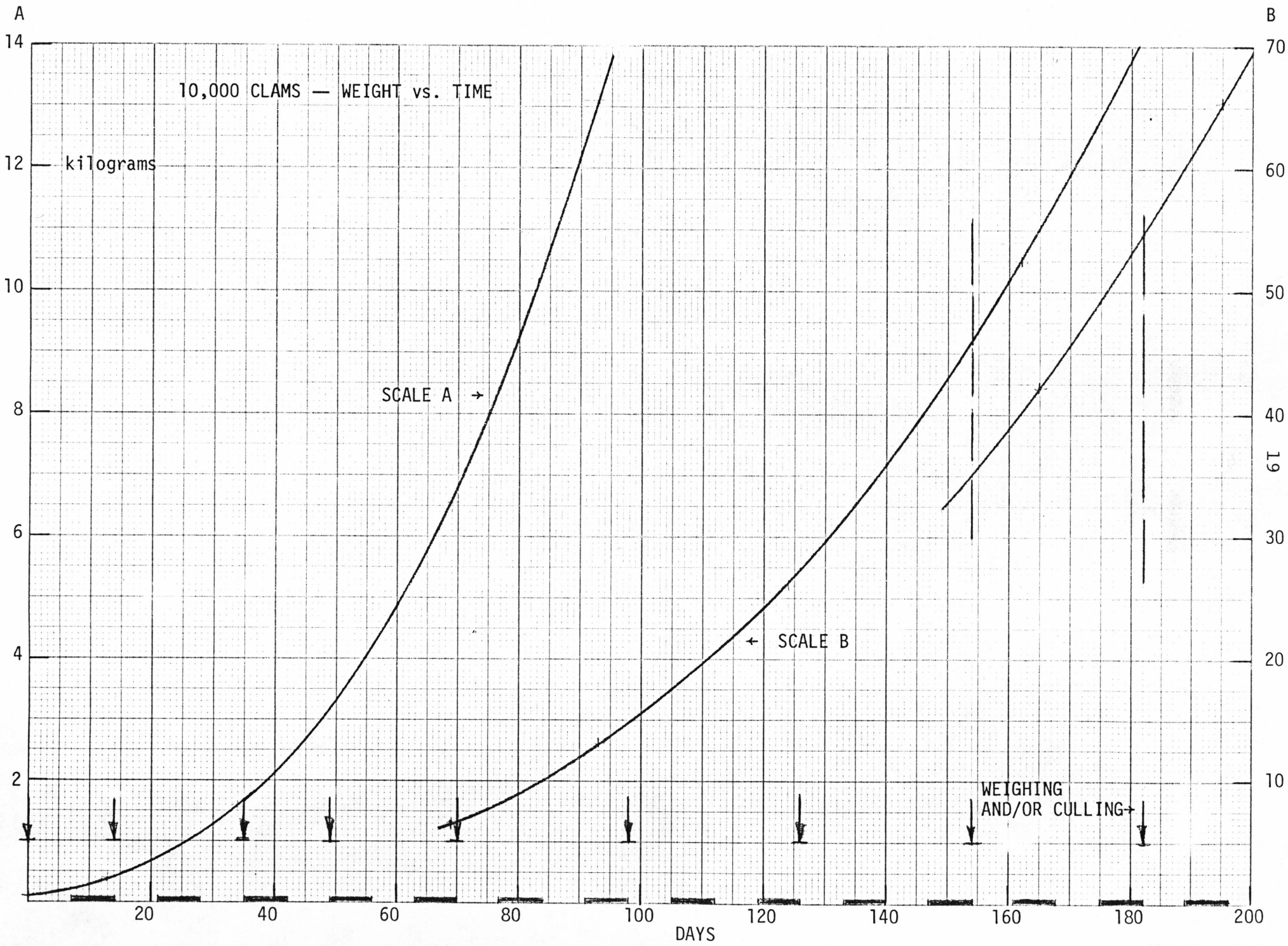


Figure 6. The predicted feeding flow required to feed a population of 10,000 clams (Tapes japonica). (In practice, the flow is adjusted stepwise.)

K+E 20 X 20 TO THE INCH • 7 X 10 INCHES
KEUFFEL & ESSER CO. MADE IN U.S.A.

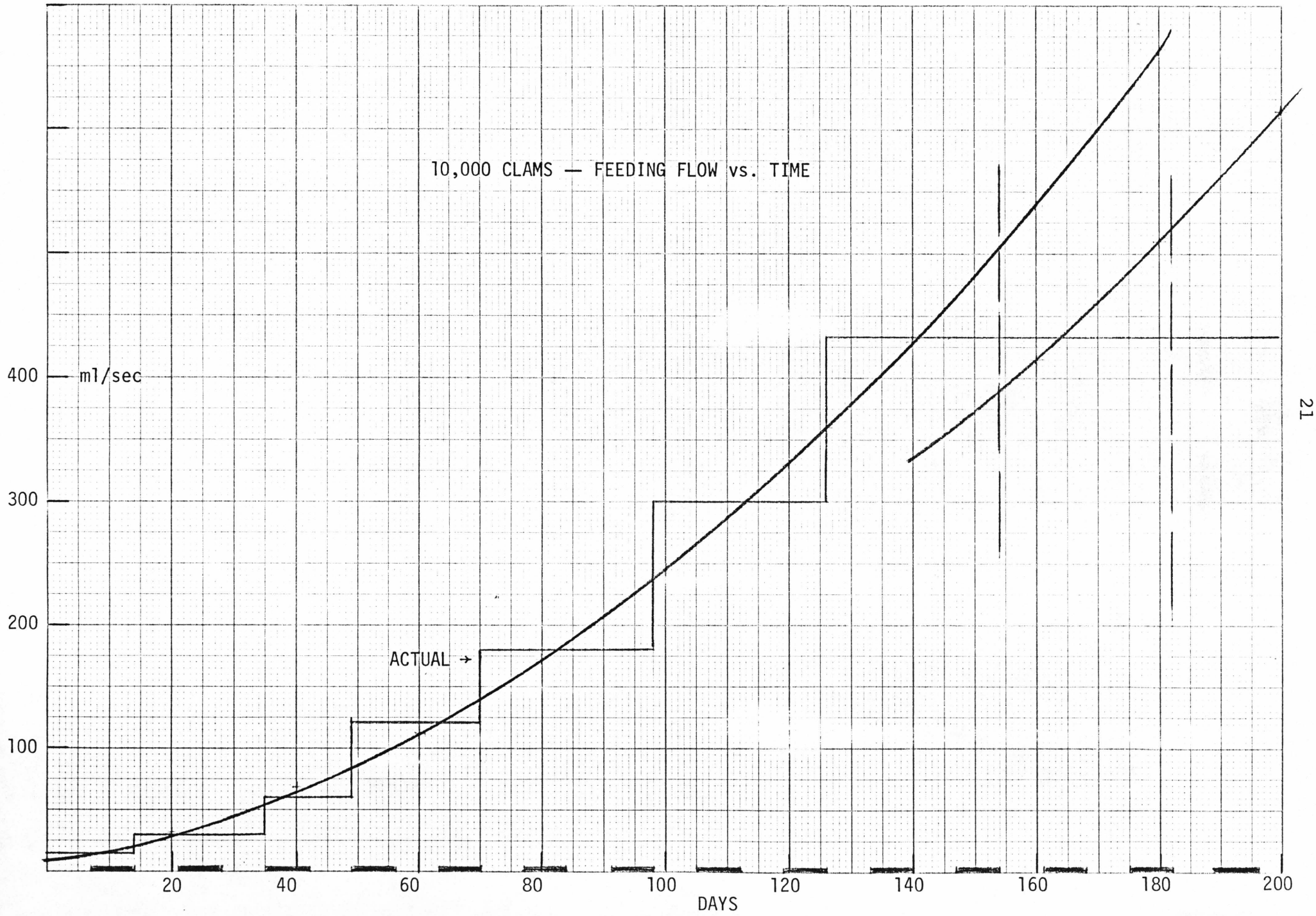


TABLE 2. FLOW RATE CHANGES

DAY	FLOW (ml sec^{-1})	SHELLFISH DIVISION
0	15	1/4 tray
14	30	1/4 tray
35	60	1/4 tray
49	120	1/4 tray
70	180	1 tray
98	300	1 tray
126 and on	110	4 trays

TABLE 3. CULLING OF EXCESS OVER PREDETERMINED WEIGHT OF SHELLFISH SUPPORTABLE BY PRESENT FOOD FLOW RATE

DAY	WEIGHTS (kg)		
	BEFORE CULLING	AFTER CULLING	CULLED
154	45.858	33.734	12.124
182	52.258	39.812	12.446
210	58.340	45.914	12.426
238	64.444	52.030	12.414
266	70.565	48.159	12.406
294	76.697	64.298	12.399
322	82.839	70.443	12.396
350	88.987	-	88.987

2.1.2.2 Maintenance

At 14 and 35 days after introduction, and after 35 days on a regular weekly schedule, the shellfish are cleaned. Half of the trays are at present on a biweekly cleaning schedule.

The shellfish tank covers are removed, the tank feedline switched off, and the accumulation of fecal material, water circulation, and general clam condition is noted.

The Nestier tray containing the shellfish is removed and placed in a shaded area while the tank is cleaned. Fecal accumulation and bacteria, if present, are noted. The effluent standpipe and main compartment chain plug are removed and the tank is drained. The aeration for the individual tank airlift recirculation system is shut off and intake fitting disconnected to drain the external recirculation system. With a hose connected to the hatchery deep-water system, the tank is rinsed and scrubbed with a stiff nylon hand-brush. The vertical sections of the external airlift recirculation system are scrubbed with a long-handled wire brush and rinsed. The silicone stopper in the airlift system is removed and the capillary tubing rinsed free of deposits. The stopper in the pool culture feed-lines is removed and the flow rate tubing cleaned by passing a smaller diameter section of tubing through it. The airlift system is then reassembled and the pool flow valve opened to allow the tank to fill while the clams are rinsed.

The clams, tray, and tray liner (if present) are rinsed with deep water to remove fecal material and the dead shellfish (if any) are removed and counted. The tray is placed back into the tank and recovered.

The individual tank/shellfish cleaning time is approximately 15 mins.

If heavy spawning occurs in the pilot plant tanks, the tanks and recirculating systems are drained after spawning is completed and the shellfish rinsed to avoid accumulation and decomposition of organic material.

2.1.2.3 Hatchery

The procedures followed in our hatchery operation are described in the hatchery operating manual (Appendix C).

2.2 Pilot Plant Observations

2.2.1 Phytoplankton

Cell counts (algae) are made twice daily on samples from the pools, the animal tanks, and the PT cultures. The purposes of the cell-counting exercise are:

(a) To follow the growth and "state-of-health" of the pools and the PTs. This information is used to verify that visual monitoring of the pools is indeed adequate. Cell counts from the pools and the PTs are plotted on semilogarithmic graph paper to obtain a graph showing the growth rates and/or yields of the algal cultures.

(b) To calculate the efficiency of the shellfish in stripping the algal cells from the pool water pumped to their tanks.

Cell counting procedures are further detailed in Appendix D.

Turbidities are taken twice daily on samples of the pools to indicate the cell densities of the cultures. A Monitek, Model 250, laboratory turbidimeter is used.

2.2.2 Shellfish

Flow rates are measured with a graduated cylinder and a stopwatch and adjusted according to instructions given by the scientist-in-charge (SIC).

2.2.2.1 Population Growth Measurements

Growth data consisting of mean individual size (length and width in mm), mean individual weight (g), total population weight (g), and estimated population number are taken when periodic transfers are made.

The individual populations are passed through a series of sieves to divide them according to size. The clams are then blotted dry and each fraction is weighed to the nearest .1 g on a Mettler balance (Model PW-1210). A random sample of 30 clams from each fraction is also removed, weighed, and measured, and this data used to determine mean clam weight, length, width, population weight, and population number per group.

As the clams become larger, the sievings are discontinued.

2.2.2.2 Allometric Growth Measurements

A subsample of 25 clams is removed from each population whenever the population is weighed (at intervals never exceeding 28 days) and the following procedure implemented:

(a) Number 25 aluminum weighing dishes and weigh same.

(b) Measure length, width, and depth of clams, to nearest .1 mm.

(c) Weigh individual clam to nearest .01 mg.

(d) Shuck meat from clam and weigh meat to the nearest .01 mg.

(e) Place shell in weighing dish and dry in oven at 60°C for 24 hr or until constant dry weight is reached.

(f) Remove aluminum dish with dried clam from oven and cool in dessicator for 1 hr.

(g) Reweigh dried clam meat and shell.

(h) Retain dried clam meat for protein analysis.

This information is used to provide a detailed analysis of shellfish growth, wet weight-dry weight relationship, and algal protein-nitrogen to shellfish meat protein-nitrogen conversion.

2.2.2.3 Spawning Record

Natural spawnings are recorded for all pilot plant tanks (shellfish manipulations and flow rate changes are noted when spawning occurs), to determine if any correlation exists.

2.2.3 Chemistry

Chemical observations performed on the pilot plant monitor the nutrient balance at different stages in the process. This involves the determination of the concentration of:

NO_2^-

NO_3^-

NH_4^+

PO_4^{---}

SiO_4^{---}

Particulate protein

Standard Technicon Autoanalyzer methods are used for the first five determinations. The particulate protein determination follows a semiautomated procedure outlined by Dorsey et al. (1977) for a heated Biuret-Folin protein assay. Samples are first digested in alkaline-copper solution (Biuret reagent), the copper-protein complex reduced by Folin-Ciocalteau phenol reagent and the molybdate blue color stabilized by cooling:

Reagents: Prepare with freshly distilled water:

1. Sodium carbonate 20% (w/v): Dissolve 200 g anhydrous sodium carbonate in and

dilute to 1 liter with glass-distilled water (GDW)

2. Sodium hydroxide (1N): Dissolve 40.0 g sodium hydroxide in and dilute to 1 liter with GDW
3. Sodium potassium tartrate 20% (w/v): Dissolve 40.0 g sodium potassium tartrate in and dilute to 200 ml with GDW
4. Copper sulfate 5% (w/v): Dissolve 10.0 g copper sulfate pentahydrate in and dilute to 200 ml with GDW
5. Phenol reagent (1N): A 1:1 dilution of commercially prepared phenol reagent, 2N (Fisher Scientific Co.)
6. Alkaline-copper solution (reagent A): Mix 10 ml sodium carbonate solution with 10 ml sodium hydroxide solution and dilute to 100 ml with GDW. Dilute 1.0 ml sodium potassium tartrate and 1.0 ml copper sulfate to 10.0 ml with GDW. Add 2.0 ml to the carbonate-hydroxide solution. This volume is sufficient for 20 samples and should be prepared daily.

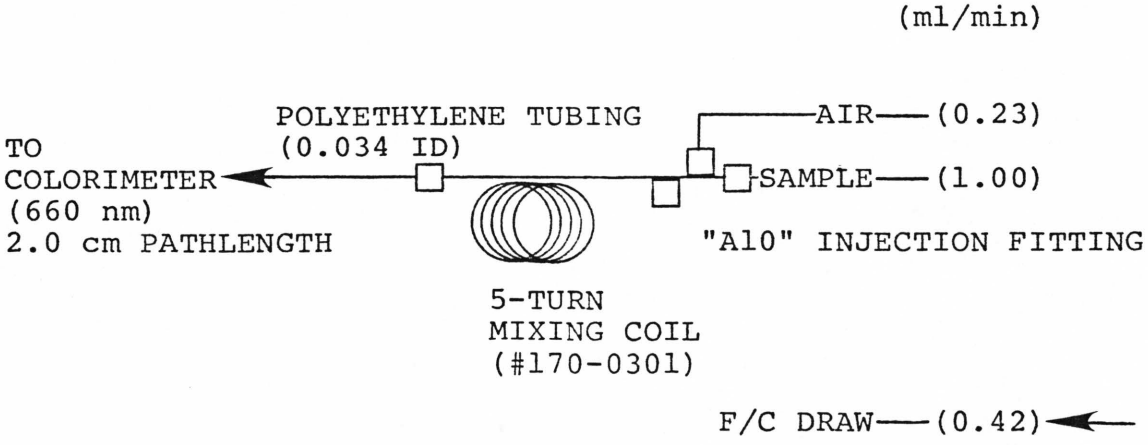
Standard:

Bovine serum albumin, crystalline. Weigh out 63 mg and dissolve in and dilute to 100 ml with 0.05N sodium hydroxide. This

is the primary standard solution containing 630 µg protein/ml.

Method: Samples may be either particulate and filtered onto 25-mm, 0.45µ glass fiber filters, or dissolved in a volume of 0.1-0.4 ml. To the sample in a 17 x 126 mm test tube, add 5.0 ml of alkaline copper solution (reagent A) with an automatic pipette and heat in a 100°C water bath for 100 mins. Remove sample from hot water bath, add 0.5 ml Folin-Ciocalteau reagent, mix immediately on a Vortex mixer, and quickly place into a 10°C cold water bath for 10-15 mins. Centrifuge at 2500 rpm for 2 mins to remove filter fibers and cell debris. Transfer the supernatant to AA-II sample cups by careful decantation or pipeting.

Protein Manifold:



Brij-35 (1 ml/liter) may be used in the sample wash to improve flow characteristics. A 30 hr^{-1}, 1:1 (wash to sample ratio) sampler cam, 2.0 cm flow cells, 660 nm interference filters at a standard calibration setting of 2.00 gives 50% full-scale deflection of recorder pen at the 20 µg protein-nitrogen level.

2.2.4 Environment

Temperatures in the polytanks are taken approximately six to eight inches from the side of the tank, just inside the air-bubble stream; in the pools, temperatures are taken from the catwalk in the area of the standpipe; in the shellfish tanks, temperatures are taken from either side of the tank about half-way down the length of the tank.

The deep-water temperature is taken from the overflow hose, which runs continuously.

Once a day, at 0800, a reading is taken from the LI-500 integrator on the beach. These numbers are then converted to kW-hr m^{-2}.

Weather, sea state, and wind speed are estimated.

All these environmental observations are recorded on a daily beach data sheet (Fig. 7).

3. DATA ANALYSIS

3.1 Pilot Plant

3.1.1 Phytoplankton

Twice daily, all active polytank inoculum cultures are sampled and cell counts performed. Similarly, both pools are sampled and cell counts plus turbidity readings taken.

The results of these observations have been collated on worksheets, of which samples are given as Tables 4 and 5.

Figure 7. All environmental observations are recorded on a beach data sheet, daily.

BEACH DATA SHEET

OBSERVER_____ WEEKDAY_____ DATE_____

WEATHER_____ SEAS_____ AIR TEMP_____ DW TEMP_____

WIND_____ RAINFALL_____

POOL LEVEL: P_1_____ REACTOR ENRICHMENT (202 ml) DW PUMP : [VACUUM_____ / FLOWRATE_____

P_2_____ R-_____ AIR PUMPS: 1)_____ 2)_____

0800

	temp	flow rate ml/10 sec	pool		temp	flow rate ml/10 sec	pool		temp
T1				T7				P1	
T2				T8				P2	
T3				T9				PT1	
T4				T10				PT2	
T5				T11				PT3	
T6				T12				PT4	

WEATHER_____ LIGHT READING

SEAS_____ 0800_____

POOL LEVEL: P_1_____ DW TEMP_____

P_2_____ DW PUMP : [VACUUM _____ / FLOWRATE_____

AIR TEMP_____

WIND_____ mph AIR PUMPS: 1)_____ 2)_____

HYPNEA TANKS:	A	B	C
0800 TEMP			
# OF CLAM TANKS			

HYPNEA TANKS:	A	B	C
1400 TEMP			
# OF CLAM TANKS			

1400

	temp	flow rate ml/10 sec	pool		temp	flow rate ml/10 sec	pool		temp
T1				T7				P1	
T2				T8				P2	
T3				T9				PT1	
T4				T10				PT2	
T5				T11				PT3	
T6				T12				PT4	

Scientist-In-Charge_____

SIC Approval_____

TABLE 4. SAMPLE OF WORKSHEETS FOR DATA COLLECTION FROM POLYTANKS

DATA LOG - ARTIFICIAL UPWELLING PROJECT - ST. CROIX (JULY 1, 1976 - OCTOBER 27, 1976)

POLY TANKS

STARTING DATE FOR ENTRIES: JULY 1, 1976

Mo-Day-Yr	Time	PT#	Algal Species	10^3 cells per ml
7-1-76	0800	1	114	540
		3	167	–
	1400	1	114	2060
7-2-76	0800	1	114	4050
		2	114	146
	1400	1	114	4260
		2	–	–
7-4-76	0800	2	167	12
	1400	2	167	22
7-5-76	0800	2	167	35
	1400	2	167	136
7-6-76	0800	2	167	162
	1400	2	167	246
7-8-76	1400	1	114	1465
7-9-76	0800	1	114	3350
		2	114	452
		4	Tet Tahiti	2
	1400	1	114	2640
		2	114	1565
		4	Tet Tahiti	2
7-10-76	0800	1	114	2230
		4	T. Tahiti	29
	1400	1	114	1390
		4	Tet Tahiti	67
7-11-76	0800	2	167	9
	1400	2	167	17
7-12-76	0800	2	167	18
	1400	2	167	–
1-20-76	0800	1	167	14
	1400	1	167	44
7-21-76	0800	1	167	96
	1400	1	167	151
7-22-76	1400	2	167	23
7-23-76	0800	2	167	49
	1400	2	167	75
7-29-76	0800	2	167	19
	1400	2	167	28
7-30-76	0800	2	167	65
	1400	2	167	67
8-2-76	0800	1	167	3
	1400	1	167	7
8-3-76	0800	1	167	7
	1400	1	167	37
8-4-76	0800	1	167	94
	1400	1	167	121
8-16-76	0800	2	167	5
	1400	2	167	1
8-17-76	0800	2	167	3
8-18-76	0800	2	167	1
		2	167	4
	1400	2	167	6
8-19-76	0800	1	167	5
		2	167	8
	1400	1	167	32
		2	167	1
8-20-76	0800	1	167	113
		2	167	–
	1400	1	167	145
		3	Tet Tahiti	22
8-21-76	0800	3	Tet Tahiti	69
	1400	3	Tet Tahiti	128
8-27-76	0800	1	167	4
	1400	1	167	23
8-28-76	0800	1	167	48
	1400	1	167	131
9-3-76	0800	2	167	4
	1400	2	167	8
9-4-76	0800	2	167	29
	1400	2	167	97
9-5-76	0800	2	167	167
	1400	2	167	185
9-6-76	0800	2	167	8
	1400	2	167	13
9-7-76	0800	1	167	37
	1400	1	167	69
9-8-76	0800	1	167	102
	1400	1	167	134
9-12-76	0800	1	167	13
	1400	1	167	30
9-13-76	0800	1	167	14
	1400	1	167	94
9-14-76	0800	1	167	127
9-17-76	0800	1	167	13
9-18-76	0800	1	167	34
	1400	1	167	19
9-19-76	0800	1	167	43
		2	167	12
	1400	1	167	73
		2	167	39
9-20-76	0800	2	167	73
	1400	2	167	96
9-24-76	0800	1	167	10
	1400	1	167	38
9-25-76	0800	1	167	51
	1400	1	167	89
9-28-76	0800	1	167	46
	1400	1	167	70
9-29-76	0800	1	167	5
	1400	1	167	19
9-30-76	0800	1	167	25
	1400	1	167	71
10-4-76	0800	1	167	6
	1400	1	167	11
10-5-76	0800	1	167	18
	1400	1	167	68
10-6-76	0800	2	167	6
	1400	2	167	12
10-7-76	0800	2	167	22
	1400	2	167	27
10-10-76	1400	1	167	10
10-11-76	0800	1	167	8
	1400	1	167	21
10-12-76	0800	1	167	37
	1400	1	167	65
10-13-76	0800	2	167	4
	1400	2	167	15
10-14-76	0800	2	167	10
	1400	2	167	18
10-18-76	0800	2	167	4
10-20-76	0800	3	167	14
	1400	3	167	15
10-21-76	0800	3	167	81
	1400	3	167	102
10-25-76	0800	1	167	16
	1400	1	167	34
10-26-76	0800	1	167	64
		2	T.150	59
	1400	1	167	75
		2	T.150	61
10-27-76	0800	3	167	2
	1400	3	167	1

DATA LOGS: STX 1019, BOOK # 20 (MAY 8, 1976 - SEPT 1, 1976)
STX 1020, BOOK # 21 (SEPT 2, 1976 - DEC. 6, 1976)

TABLE 5. SAMPLE OF WORKSHEETS FOR DATA COLLECTION FROM POOLS

DATA LOG - ARTIFICIAL UPWELLING PROJECT, ST. CROIX (NOV. 5, 1976 - NOV. 25, 1976)

POOL 1

Mo-DAY-YR	Time	Algal Species	10^3 cells per ml	pool Status	pool turbidity ppm	pool protein mg/L	pool protein µg-at N/L	pool NO_3+NO_2 µg-at N/L	pool NH_4-N µg-at N/L	pilot plant
11-5-76	0800	167	48	ON	5.6					+
	1400	167	35	ON	8.5					+
11-6-76	0800	167	24	ON	4.6					+
	1400	167	33	ON	7.2					
11-7-76	0800	167	6	ON	2.7					
	1400	167	0	ON	1.7		9.08			
11-8-76	0800	167	—	ON	—					
	1400	167	—	ON	—					
11-9-76	0800	167	—	SCRUB	—					
	1400	167	—	INOC	—					
11-10-76	0800	167	12	OFF	3.0					
	1400	167	13	OFF	3.6		13.64			
11-11-76	0800	167	29	OFF	6.2					
	1400	167	42	ON	18.0					
11-12-76	0800	167	59	ON	7.6					+
	1400	167	65	ON	10.5	2390	26.20			+
11-13-76	0800	167	56	ON	9.2					+
	1400	167	79	ON	14.9					+
11-14-76	0800	167	70	ON	9.2					+
	1400	167	55	ON	11.5					+
11-15-76	0800	167	75	ON	9.6	2010	22.92			+
	1400	167	77	ON	10.1					+
11-16-76	0800	167	55	ON	7.5					+
	1400	167	58	ON	11.2					+
11-17-76	0800	167	50	ON	6.2					+
	1400	167	54	ON	8.4	2250	25.74			+
11-18-76	0800	167	55	ON	12.1					+
	1400	167	62	ON	7.9					+
11-19-76	0800	167	52	ON	6.0	1640	18.77	$3.96 \pm .05$	1.76 ± 0.16	+
	1400	167	56	ON	8.3					+
11-20-76	0800	167	30	ON	4.7					+
	1400	167	37	ON	8.4					+
11-21-76	0800	167	26	ON	3.8					+
	1400	167	37	ON	5.2					+
11-22-76	0800	167	23	ON	4.2					+
	1400	167	28	ON	5.4	1380	15.81	*ND	0.19 ± 0.05	+
11-23-76	0800	167	21	ON	2.6					+
	1400	167	51	ON	6.3					+
11-24-76	0800	167	35	ON	4.6	920	10.52	$0.75 \pm .06$	2.2 ± 0.11	+
	1400	167	45	ON	5.4					+
11-25-76	0800	167	24	ON	3.7					+
	1400	167	35	ON	4.6					+

* ND = NOT DETECTABLE

POOL 2

Algal Species	10^3 cells per ml	pool Status	pool turbidity ppm	pool protein mg/L	pool protein µg-at N/L	pool NO_3+NO_2 µg-at N/L	pool NH_4-N µg-at N/L	pilot plant
167	49	ON	10.8					
167	44	ON	20.0					
167	70	ON	12.5					
167	63	ON	15.5					+
167	59	ON	9.0					+
167	68	ON	10.5		22.61			+
167	58	ON	7.7					+
167	37	ON	10.5					+
167	41	ON	6.6					+
167	29	ON	11.9					+
167	32	ON	5.6					+
167	42	ON	6.6		22.49			+
167	34	ON	4.5					+
167	38	ON	6.4					+
167	27	ON	9.1					
167	24	ON	7.2	1590	18.20			
167	20	ON	3.9					
167	35	ON	5.8					
167	21	ON	4.0					
167	26	ON	5.7					
167	24	ON	3.8	1120	12.76			
167	21	ON	5.7					
167	18	ON	5.8					
167	18	ON	6.6					
167	14	ON	5.0					
167	36	ON	5.8	1870	21.39			
167	34	ON	4.6					
167	29	ON	6.4					
167	25	ON	4.4	1280	14.57	$7.65 \pm .05$	2.11 ± 0.16	
167	24	ON	6.6					
167	21	ON	4.6					
167	44	ON	8.6					
167	20	ON	4.6					
167	37	ON	5.2					
167	19	ON	4.2					
167	28	ON	6.0	1380	15.81	$0.97 \pm .05$	$2.48 \pm .05$	
167	22	ON	3.9					
167	35	ON	6.1					
167	18	ON	3.7	900	10.29	$10.71 \pm .06$	$2.71 \pm .11$	
167	45	ON	6.4					
167	19	ON	3.0					
167	33	ON	4.4					

DATA LOGS:
CELL COUNTS - STX 1020 BOOK 21 (SEPT. 2, 1976 - DEC. 6, 1976)
TURBIDITY - STX 1320 (FEB. 16, 1974 - JAN 30, 1977)
CHEM NUTRIENTS PROTEIN DATA - STX 1349
PILOT PLANT PROTEIN SAMPLING LOG - STX 1292

3.1.2 Shellfish

The shellfish were introduced into the pilot plant according to the schedule in Table 6.

At the time of actual introduction, Batches #20 and #21 had exceeded the planned-for introduction size. The corrected date corresponds to the day on which the planned introduction size is considered to have been reached. This is the date used for subsequent feeding management.

It should be noted that these dates are at 50, 57, and 56 days after spawning. A 45-day span had been anticipated based on extrapolation of our feeding experiment growth data. The observed discrepancy can be attributed entirely to a different growth rate before the larvae "set."

The pilot plant shellfish weight increases since corrected introduction date are given in Table 7.

There is a discrepancy between "expected weight" and "measured weight." The cause of this discrepancy is not identified, but differences between the present pilot plant operation and the 36-day feeding study upon which the "expected weight" was based lead to the following possibilities:

- overfeeding
- higher packing density in the pilot plant
- vertical food flow through the shellfish bed
- recirculation
- single diatom as opposed to mixed diet
- individual size is larger now in pilot plant than during the 36-day feeding experiment

TABLE 6. INTRODUCTION OF SHELLFISH INTO THE PILOT PLANT

BATCH #	TRAY #	DATE OF INTRODUCTION		
		SCHEDULED	ACTUAL	CORRECTED*
20	1	09/01/76	10/13/76	09/22/76
21	5	09/21/76	10/15/76	10/12/76
22	9	10/11/76	10/19/76	10/19/76

*Note: According to our plan of work as described in the section 2.1.2 (page 20) of our proposal for 1976-77, we intended to introduce the shellfish into the pilot plant when they had reached an average length of 4.3 mm and a weight of .01 g.

TABLE 7. PILOT PLANT SHELLFISH WEIGHT INCREASES SINCE CORRECTED INTRODUCTION DATE

DAYS IN SYSTEM	**EXPECTED WEIGHT (kg)	MEASURED WEIGHT (kg)		
		POPULATION 1 (TRAYS 1-4)	POPULATION 2 (TRAYS 5-8)	POPULATION 3 (TRAYS 9-12)
14	.400	-	.333	.935
35	1.600	1.340	1.339	2.100
70	6.4	3.5	3.5	4.7
98	14.827	7.348	8.376	9.414
126	27.480	10.705*	13.270*	13.490*
154 Δ	45.858	15.660*	19.650*	19.530*
154 ∇	33.734	15.660*	19.650*	19.530*
182 Δ	52.258	19.720*	21.100*	23.420*
182 ∇	39.812	19.720*	21.100*	23.420*
196	48.486	22.760*	- not available yet -	

*Distributed among four trays.
**Expected growth was derived from our Nov/Dec 1976 feeding experiment.
ΔBefore culling.
∇After culling.

- temperature
- irregularity of food-flow concentration
- increased spawning in the pilot plant
- additions provided to shellfish from supplements to one of the phytoplankton cultures

An intensive analysis of cell data collected on the pilot plant during its first seven months of operation is underway, and preliminary conclusions are that the causes of discrepancy between expected and actual weight increases will be identified and that changes in the feeding criteria will lead to improved growth over the upcoming year.

3.1.3 Chemistry

The recorded values for deep-sea water inorganic "nitrogen" are listed in Table 8.

Protein-nitrogen assays, performed on the system at different stages, are presented in Table 9.

3.2 Developmental Research

3.2.1 Species Selection for Growth in Unsupplemented Deep Water

During the first quarter of this year, five algal species were selected for testing in continuous culture after demonstrated ability to grow in batch culture of unenriched deep water. Of these clones, three were isolated at the St. Croix station: STX-19, STX-183 and STX-97. These clones, along with WHOI-581 and Tetraselmis tetrathele, were all tested in reactor cultures at a dilution rate of 1.0

TABLE 8. DEEP WATER INORGANIC "NITROGEN" MEAN + STANDARD DEVIATION VALUES (µg-at/l)

DATE	READING	DATE	READING	DATE	READING
07/01/76	33.94*	11/04/76	29.32	03/02/77	31.95
	2.25		0.89		n.a.
07/08/76	33.23*	11/11/76	28.38	03/09/77	31.85
	0.32		2.00		n.a.
07/15/76	33.34*	11/18/76	30.11	03/16/77	34.29
	0.13		1.01		n.a.
07/22/76	33.19*	11/25/76	32.05	03/23/77	30.75
	0.39		1.77		n.a.
07/30/76	33.71*	12/02/76	30.18	03/30/77	32.25
	0.89		0.60		n.a.
08/06/76	33.21*	12/10/76	31.50	04/06/77	33.32
	0.61		1.14		n.a.
08/13/76	28.99*	12/17/76	32.45	04/13/77	32.61
	1.68		1.04		n.a.
08/28/76	33.22*	12/22/76	31.53	04/20/77	33.91
	0.41		0.39		n.a.
09/10/76	33.78*	12/31/76	31.16		
	0.30		1.26		
09/16/76	33.58*	01/05/77	29.82		
	0.12		0.61		
09/23/76	33.73*	01/12/77	28.72		
	0.16		0.93		
10/01/76	28.97*	01/19/77	30.53		
	1.01		0.53		
10/07/76	29.58	01/26/77	30.87		
	0.52		1.36		
10/15/76	28.92	02/02/77	30.09		
	1.50		0.51		
10/22/76	28.92	02/16/77	29.84		
	0.48		0.66		
10/28/76	27.43	02/23/77	31.62		
	1.38		n.a.		

*NO_3^- + NO_2^- only. No NH_4^+ readings available.

TABLE 9. POOL PHYTOPLANKTON PROTEIN-N PRODUCTION, AND DEEP-WATER "N" → PHYTOPLANKTON PROTEIN-"N" CONVERSIONS

Culture No.	Pool No.	Date Pool On (1400 Hrs)	Date Pool Off (0800 Hrs)	Hours On (Actual)	Hours On (Predicted)	Hours P-A	Deep Water Dissolved "N" Date	Deep Water Dissolved "N" $\mu gatL^{-1}$	Phytoplankton Protein-N Date	Phytoplankton Protein-N $\mu gatL^{-1}$	Dw "N"→Phyto Protein-N (%) All data	Dw "N"→Phyto Protein-N (%) Less non-steady state
1	1	9/26/76	10/12/76	378	666	288	9/23	33.73	9/29	28.46		
							10/1	28.79	10/2	26.86		
							10/7	29.58	10/5	47.77		
							10/15	28.92	10/8	13.31		
							μ =	30.26	μ =	29.10	96	76
									μss =	22.88		
2	2	9/21/76	10/7/76	378	666	288	9/23	33.73	9/29	27.77		
							10/1	28.79	10/2	23.54		
							10/7	29.58	10/5	10.46		
							μ =	30.70	μ =	20.59	67	67
3	2	10/8/76	10/20/76	282	666	384	10/7	29.58	10/8	04.74		
							10/15	28.92	10/14	24.46	57	77
							μ =	29.14	μ =	16.51		
									μss =	22.40		
4	1	10/13/76	10/26/76	306	666	360	10/15	28.92	10/14	17.26		
							10/22	28.92	10/20	23.02		
							10/28	27.43	10/23	05.13		
							μ =	28.42	μ =	15.14	53	71
									μss =	20.14		
5	2	10/23/76	11/2/76	234	666	432	10/22	28.92	10/23	23.21		
							10/28	27.43	10/26	26.45		
							11/4	29.32	10/29	17.98		
									11/1	02.04		
							μ =	28.56	μ =	17.42	61	79
									μss =	22.55		

Culture No.	Pool No.	Date Pool On (1400 Hrs)	Date Pool Off (0800 Hrs)	Hours On (Actual)	Hours On (Predicted)	Hours P-A	Deep Water Dissolved "N" Date	Deep Water Dissolved "N" $\mu gatL^{-1}$	Phytoplankton Protein-N Date	Phytoplankton Protein-N $\mu gatL^{-1}$	Dw "N"→Phyto Protein-N (%) All data	Dw "N"→Phyto Protein-N (%) Less non-steady state
6	1	10/28/76	11/9/76	282	666	384	10/28	27.43	10/29	24.55		
							11/4	29.32	11/1	22.97		
							11/11	28.38	11/4	21.90		
									11/7	09.08		
							μ =	28.38	μ =	19.63	69	82
									μss =	23.14		
7	2	11/4/76	11/26/76	522	666	144	11/4	29.32	11/4	28.71		
							11/11	28.38	11/7	22.61		
							11/18	30.11	11/10	22.49		
							11/25	32.05	11/12	18.20		
							μ =	29.97	11/15	12.76		
									11/17	21.39		
									11/19	14.57		
									11/22	15.81		
									11/24	10.29		
									μ =	18.54	62	62
8	1	11/11/76	11/30/76	450	666	216	11/11	28.38	11/12	26.20		
							11/18	30.11	11/15	22.92		
							11/25	32.05	11/17	25.74		
							μ =	30.18	11/19	18.77		
									11/22	15.81		
									11/24	10.52		
									11/26	08.96		
									11/29	06.56		
									μ =	16.94		
									μss =	19.99	56	66
9	2	11/29/76	12/16/76	402	666	264	12/2	30.18	11/29	18.82		
							10/10	31.50	12/1	26.98		
							12/17	33.37	12/3	15.67		
							μ =	31.68	12/6	23.68		
									12/8	19.07		
									12/10	24.14		
									12/13	27.40		
									12/15	15.14		
									μ =	21.36	67	67

Culture No.	Pool No.	Date Pool On (1400 Hrs)	Date Pool Off (0800 Hrs)	Hours On (Actual)	Hours On (Predicted)	Hours P-A	Deep Water Dissolved "N" Date	Deep Water Dissolved "N" $\mu gatL^{-1}$	Phytoplankton Protein-N Date	Phytoplankton Protein-N $\mu gatL^{-1}$	Dw "N"→Phyto Protein-N (%) All data	Dw "N"→Phyto Protein-N (%) Less non-steady state
10	1	12/2/76	12/23/76	498	666	168	12/2	30.18	12/3	19.93		
							12/10	31.50	12/6	28.12		
							12/17	33.37	12/8	25.10		
							12/22	31.53	12/10	30.65		
							μ	= 31.65	12/13	29.41		
									12/15	29.04		
									12/17	15.81		
									12/20	17.82		
									12/22	08.75		
									μ	= 22.71	72	77
									μss	= 24.45		
11	2	1/18/76	1/6/77	450	666	216	12/22	31.53	12/20	30.14		
							12/31	31.16	12/22	27.22		
							1/5	29.82	12/24	21.70		
							μ	= 30.84	12/27	23.67		
									12/29	20.69		
									12/31	22.64		
									1/3	09.90		
									1/5	04.34		
									μ	= 20.04	65	79
									μss	= 24.34		
12	1	12/26/76	1/11/77	378	666	288	12/31	31.16	12/27	21.31		
							1/5	29.82	12/29	18.26		
							1/12	28.78	12/31	28.48		
							μ	= 29.92	1/3	24.02		
									1/5	28.17		
									1/7	21.02		
									1/10	22.72		
									μ	23.43	78	78

Culture No.	Pool No.	Date Pool On (1400 Hrs)	Date Pool Off (0800 Hrs)	Hours On (Actual)	Hours On (Predicted)	Hours P-A	Deep Water Dissolved "N" Date	Deep Water Dissolved "N" µgatL^{-1}	Phytoplankton Proetin-N Date	Phytoplankton Proetin-N µgatL^{-1}	Dw "N"→Phyto Protein-N (%) All data	Dw "N"→Phyto Protein-N (%) Less non-steady state
13	2	1/8/77	1/25/77	402	666	264	1/5	29.82	1/10	30.56		
							1/12	28.78	1/12	30.96		
							1/19	30.58	1/14	26.59		
							1/26	30.87	1/17	29.80		
							µ	= 30.01	1/19	23.98		
									1/21	23.31		
									1/24	03.43		
									µ	= 24.09	80	92
									µss	27.53		
14	1	1/13/77	2/8/77	618	666	48	1/12	28.72	1/14	26.47		
							1/19	30.58	1/17	29.30		
							1/26	30.87	1/19	23.64		
							2/2	30.09	1/21	31.55		
							2/9	30.16	1/24	26.58		
							µ	= 30.08	1/26	29.82		
									1/28	29.06		
									1/31	30.63		
									2/2	28.73		
									2/4	29.24		
									2/7	31.06		
									µ	= 28.73	96	96
15	2	1/27/77	2/22/77	618	666	48	1/26	30.87	1/27	27.75		
							2/2	30.09	1/31	30.54		
							2/9	30.16	2/2	29.93		
							2/16	29.84	2/4	28.72		
							2/23	32.25	2/7	33.40		
							µ	= 30.64	2/9	32.19		
									2/11	23.96		
									2/14	37.03		
									µ	= 29.82	97	97
16	1	2/10/77	3/10/77	666	660	0	-	-	-	-		

turnovers per day. STX-19 and STX-183 washed out at a rate greater than the dilution rate, indicating that not only did they not divide fast enough but were dying off as well. STX-97, also tested in the same conditions, washed out at a slower-than-dilution rate, indicating that it may grow successfully at a lower dilution rate. It was later grown in reactor cultures at .25, .50, .75 and 1.0 dilutions/day. Results using these dilution rates were erratic; growth did occur, but was unpredictable. WHOI-581 was abandoned because it is not a good food for juvenile shellfish.

T. tetrathele, a flagellate clone received 9/5/76 from Tahiti, was tested in 2000-liter outdoor containers and was found to grow equally well in enriched and unenriched deep water. It could be maintained at a steady state over a range of .25-1.0 dilutions/day in unsupplemented deep water. However, the cultures could not be maintained unialgally for more than one week. Unidentified diatoms took over in all cases. This alga may be useful sometime in the future for hatchery work where larval food is needed for a short period of time.

A high-temperature Isochrysis sp., clone T-Iso, also received from Tahiti (CNEXO), was started in a one-month outdoor continuous flow experiment at .50 dilutions/day. Identical tanks were employed, one enriched with chelated trace metals and vitamins, the other completely unsupplemented deep water. Cell production averaged three times higher in the enriched cultures than in the cultures without

enrichment (see Table 10). At this point it was noted that washout did not uccur in any of these cultures, nor were any other organisms observed in routine counting. In subsequent experiments, T-Iso was successfully grown at densities averaging 100,000 cells/ml in unsupplemented reactor cultures. These cultures remained at steady state when terminated after six weeks. Dilution rates were .25 and .75 turnovers/day.

Although production of T-Iso is somewhat lower than STX-167, its greater stability and its adaptability for larval and mature shellfish make it attractive for large-scale use in the St. Croix mariculture system. Recently, a naked flagellate, designated STX-190, was isolated from the deep water pipelines. It has grown well in F/2-medium batch culture and is presently being tested in continuous culture on unsupplemented deep water. Data for this clone will be included in the final report.

3.2.2 Stability of Phytoplankton Cultures

Pool collapses are typified by a decline in cell density much faster than the dilution rate, indicating that cessation of growth cannot be the only cause for the collapses (in the absence of growth alone, cell density decreases would match the dilution rate). The diatom cells are being killed by some agent, or are being removed from the population (as by grazing), or both; cessation of growth of existing cells may also be occurring.

Possible causes have been classified into the following four categories:

TABLE 10. EFFECT OF ENRICHMENT OF DEEP WATER ON GROWTH OF CLONE T-Iso IN CONTINUOUS-FLOW OUTDOOR REACTOR CULTURES AT .50 DILUTIONS/DAY

	CELL DENSITY*	
	10^4 CELLS/ml	(±1 S.D.)
Plus Enrichment*		
Reactor 17	21.7	(±19.1)
Reactor 20	9.8	(± 9.6)
Average:	15.7	
No Enrichment		
Reactor 18	4.6	(± 4.0)
Reactor 19	4.4	(± 4.3)
Average:	4.5	

*Enrichment includes the trace metals Fe, Cu, Ca, Mn, Mo, and Zn, and vitamins B_{12} and B_1.

**Cell densities given are averages of 26 daily measurements made in duplicate for each culture at 0800 hr.

(a) Toxicity of dissolved compounds in the pool.

(b) Interaction with other organisms.

(c) Changes in our laboratory stock cultures of STX-167.

(d) Environmental factors.

A multi-pronged plan of work has been drawn up and is partly implemented. The experiments undertaken or planned are summarized as follows:

(a) Interaction between lab cultures and pool samples taken from a "collapsed" pool. This type of experiment is performed with filtered or unfiltered pool samples.

(b) Chemical supplementation of pool samples followed by reinoculation.

(c) Qualification and quantification of biological contaminants in pool cultures.

(d) Observation of cell size evolution.

(e) Use of other clones of Chaetoceros curvisetus.

(f) Evaluation of the correlation between environment and "collapse".

In five laboratory batch experiments lasting 5-10 days with collapsed pool cultures of C. curvisetus, STX-167, we have demonstrated that addition of nutrients and/or reinoculation of raw pool water with C. curvisetus from the lab cultures does not result in growth of the diatom. When the collapsed pool culture water is filtered to remove all cells larger than bacteria, the water can support growth of the diatom, but the growth is sometimes killed later by

secondary infection by bacteria. The effect is more obvious and drastic in flasks where excess nutrients are added. The bacterial flora which develops in such batch cultures, with or without added nutrients, is not considered to be a cause for collapse of the continuously diluted pool cultures. Future experiments will consist of frequent observations of manipulated cultures, so as to observe the sequence of events prior to the onset of secondary infections. In one such experiment during March 14-17, 1977, rapid die-off of C. curvisetus was accompanied by rapid increase in numbers of other organisms (flagellates and protozoa). We are maintaining a culture of this mixed population and hope to use it in further experiments with healthy diatom cultures. We are also getting help from outside our group in identifying and culturing the organism(s) causative (perhaps) of collapse.

We are continuing to attempt to isolate a new C. curvisetus strain, but none has grown in our daily deep-water samples.

Another type of experiment in which semipermeable membranes are used to separate sterile lab culture from collapsed pool culture will be done in the laboratory. An outdoor experiment was attempted but was unsuccessful because of the difficulty in working outdoors with the experimental culture vessel.

3.2.3 Possible Antibiotic Production by an Algal Flagellate

Outdoor 200-gallon batch cultures of T-Iso

have been maintained constantly since November 19, 1976, in enriched seawater medium F/2 minus Si. These cultures have remained virtually free of contaminating organisms (bacteria, other algae and prctozoans), non-bacterial contaminants averaging less than 0.02% of cell counts. Normally, batch cultures of diatoms and other flagellates become heavily contaminated following exponential growth phase (usually after 3-4 days), making the cultures useless as innocula for pool or reactor cultures. In contrast, batches of T-Iso have repeatedly grown without any manipulation for over two months, maintaining an average cell density of 5×10^6 cells/ml.

A possible explanation for the apparent resistance of cultures of clone T-Iso to invasion by other organisms is that it excretes a substance which is toxic to other organisms (an antibiotic). This was tested by culturing marine bacteria and three other clones of microalgae in the 0.5 µm-filtered supernatant taken from the outdoor culture when it was two weeks old. Growth of the mixed marine bacteria population, and of two of the algae tested (a flagellate and a diatom) was inhibited; growth of a green algal flagellate and of the producer (T-Iso) was not inhibited (Table 11).

Recently, a first test of cell extracts of lab-grown T-Iso, in collaboration with Mrs. Helen Gjessing of the College of the Virgin Islands, St. Croix, showed no antibiotic activity with pathogenic bacteria. The test is being repeated. Similar experiments with marine bacteria gave

TABLE 11. GROWTH OF TEST BACTERIA AND ALGAE IN MEDIUM F/2 IN THE SUPERNATANT FROM A TWO-WEEK-OLD T-Iso CULTURE

TEST ORGANISM	GROWTH IN T-Iso SUPERNATANT AS % OF GROWTH IN DEEP-WATER CONTROLS	
Mixed marine bacteria	50.7	(±1.7)
S-1 (flagellate)	53.0	(±4.3)
STX-114 (diatom)	85.6	(±2.3)
STX-92 (green flagellate)	105.8	(±4.1)
T-Iso	126.9	(±2.3)

Note: Values are average yields of duplicate cultures in T-Iso supernatant, expressed as a percentage of yields observed in duplicate control cultures in F/2 medium made from deep water. Values in parentheses are ±1 standard deviation. Growth of bacteria was measured as turbidity; growth of algae by means of cell counts.

similar results. Although these results are somewhat disappointing from a pharmacological view, the importance of T-Iso to shellfish mariculture is not diminished when considering the 49% inhibition of marine bacteria from cell-free supernatant and its impressive stability in beach experiments. We are continuing to explore possibilities in both fields.

All results relevant to developmental research on microalgae will be presented in detail in the final report to the agency.

3.2.4 Diet Testing

A preliminary diet test was conducted with Isochrysis sp., a flagellate clone isolated from Tahiti (T-Iso), on Tapes japonica larvae (Batch #24). The survival and growth of larvae fed a monoculture diet of T-Iso was compared to identical concentrations of Tapes Batch #24 larvae fed Bellerochea polymorpha (STX-114) and Thalassiosira pseudonana (3H). Through metamorphosis to Day 25, the larvae were maintained in 15-liter buckets, filtered and fed every other day (antibiotic Streptomycin sulfate added), and transferred to 40-liter flumes on Day 15 to set.

Results from the preliminary test are shown in Tables 12(a) and (b).

Critical growth of the larvae fed T-Iso was more rapid than the larvae fed STX-114 and 3H; however, little growth occurred after Day 8. The T-Iso larvae failed to reach setting size by Day 30.

TABLE 12. GROWTH AND SURVIVAL OF SHELLFISH LARVAE (Tapes japonica) FED TWO DIFFERENT DIETS

(a) DIET: STX-114 and 3H

DAY	LARVAE LENGTH AND WIDTH (μ)	% SURVIVAL
4	114 x 91	60
8	155 x 135	43
10	171 x 151	43
15	215 x 198	40
25	229 x 223	39
30	233 x 221	13

(b) DIET: Tahiti Isochrysis (T-Iso)

DAY	LARVAE LENGTH AND WIDTH (μ)	% SURVIVAL
4	130 x 108	75
8	165 x 145	63
10	169 x 149	63
15	169 x 156	23
25	186 x 170	23
30	186 x 170	13

Growth and survival of larvae fed the diet of STX-114 and 3H was average through setting (up to Day 15), but decreased markedly thereafter. The survival of both groups to Day 30 was poor.

A second diet test was conducted to determine the survival and growth of replicate larval concentrations fed T-Iso and 3H or STX-114 (depending on which was available), and larvae fed S-1 (another unidentified naked flagellate) and 3H or STX-114. Food concentrations were proportioned, as determined by culture turbidity samples, to provide a 1×10^5 ml^{-1} cell concentration. The same larval culturing techniques were followed as in the first diet test.

The results of the second diet test are shown in Tables 13(a) and (b).

Growth and survival of both groups of larvae were extremely poor and the experiment was terminated on Day 8.

No valid conclusions may be drawn concerning the quality (as food for shellfish) of T-Iso. The poor survival of larvae in both experiments may be attributed to bacterial immunity to the present antibiotics, or to increased bacterial concentration in the hatchery plumbing.

In an effort to increase larvae survival, new antibiotics at various dosage levels are being tested and deep-water lines in the hatchery are on continuous flow. When the larvae survival rate is improved, additional diet tests will be conducted to evaluate the nutritional value of T-Iso.

TABLE 13. GROWTH AND SURVIVAL OF SHELLFISH LARVAE (Tapes japonica) FED TWO DIFFERENT DIETS

(a) DIET: S-1 and 3H or STX-114

DAY	LARVAE LENGTH AND WIDTH (μ)	% SURVIVAL
2	106 x 85	73
4	110 x 90	31
6	115 x 93	18
8	112 x 93	7

(b) DIET: T-Iso and 3H or STX-114

DAY	LARVAE LENGTH AND WIDTH (μ)	% SURVIVAL
2	98 x 77	66
4	106 x 83	22
6	112 x 92	12
8	114 x 94	6

3.2.5 Large Clam Feeding Study

An experiment was designed to evaluate the quantity of food necessary for sustained growth of larger Tapes japonica (>25 mm shell length) and to evaluate the pilot plant tank design.

Food for the shellfish was supplied by one reactor culture. Influent and effluent protein samples were taken three times per week. The shellfish were weighed weekly (total wet weight) and a random sample of 30 clams taken to determine mean individual shellfish length and weight. Mortality was also recorded. Flow rate changes were based on weekly population weight increases.

In a final phase of this study, the tank was converted to a once-through system by turning off the airlift aeration and stoppering the recirculation ports. An airline was placed in the influent tank chamber to increase the dissolved oxygen concentration of the culture flow. After six weeks the airline was removed and the study continued for two weeks with no additional aeration. The same population of clams was used throughout the study.

Population weight increases, mortality, available phytoplankton protein-N, and stripping efficiencies were compared for shellfish on a recirculation system, once-through with aeration, and once-through without aeration.

A group of 251 clams with a total starting weight of 2064.8 g was introduced on Day 0. Mean clam size (length) ranged from 29.5 mm (SD=2.3) to 35.9 (SD-3.4) throughout the study period.

The mean population weight increase for the shellfish in the recirculation design system was 12.77 g over 13 weeks compared to 20.50 g over 6 weeks for the once-through system with aeration, and 20.3 g over 2 weeks for the once-through system without aeration.

Shellfish mortality in the recirculation system was 7%. Mortality in the once-through system without aeration and with aeration was 1%.

The mean available phytoplankton protein-N ($\mu g\text{-at } l^{-1} wk^{-1}$) for the recirculation system was 12.53 as compared to 9.61 and 9.20, respectively, for the once-through with aeration and once-through without aeration systems.

Weekly phytoplankton stripping efficiencies were 53.1% in recirculation flow, and 71.4 and 68.1%, respectively, for the once-through with aeration and once-through without aeration systems.

In general, less phytoplankton protein-N was available for the shellfish during the once-through flow period of the experiment; however, better shellfish growth and stripping was observed during the once-through periods as compared to the recirculation period. Mortality was significantly higher during the recirculation flow period.

Observations made during the study indicate that fecal material collects in areas of poor water flow in both the recirculation and once-through designs, instead of remaining in suspension or being carried out. Thus, the possibility of bacterial contamination is increased. The

recirculation of phytoplankton culture back through the shellfish did not increase the stripping efficiency of phytoplankton by shellfish, as expected. Frequent spawnings were also observed before and after cleaning of shellfish during the recirculation study period.

Because of the different durations of each phase of this study, it is not possible to treat all results equally. This preliminary study does indicate, however, that it may be possible to decrease operational costs by eliminating flow recirculation and modifying the present tank design.

3.2.6 Tapes Comparative Growth Study —Pilot Plant vs. Flume

The present pilot plant operation incorporates a new tank design with an airlift recirculation system in which the culture mixture flows underneath and up through the clams, rather than over and across them, as in previous shellfish tank designs. The comparative growth study of the pilot plant Tapes vs. an identical population reared in trays in a hatchery flume was designed to determine if there were gross differences in shellfish growth rates due to tank design.

Due to food constraints, the Tapes flume populations (initially size and weight groups identical to those in the pilot plant) were reduced by 75%, with matched populations in the pilot plant. The flume populations received a total STX-167 reactor culture flow of 46 ml/10 sec (at

approximately 10^5 cells/ml) and the populations were rotated in the flume weekly. After distribution into four trays, the pilot plant populations received 110 ml/sec/tray of STX-167 pool cultures (approximately 1 x 10^5 cells/ml). The flume populations are cleaned weekly, weighed when their corresponding pilot plant populations are weighed, and mortality recorded.

The most recent data on individual clam size and weight and total population weight are represented in Table 14.

All pilot plant populations are larger (mean individual length) and weigh approximately twice as much as the flume population clams.

Significant data were available to determine the volume of food (ml) available per second per clam in the flume and pilot plant populations. The results are shown in Table 15. The pilot plant populations are receiving an average of .05 ml/sec/clam or five times more food than the flume population's .01 ml/sec/clam.

Total mortality to-date (early May) for the flume and pilot plant populations are given in Table 16. Mortality is significantly greater in all pilot plant tanks than in the coresponding flume populations. Percent mortality figures for the pilot plant and flume populations' batches #20, 21, and 22, were 18%, 18%, 14% in the pilot plant and 5%, 3%, 7% in the flume.

Spawning continues to be a problem in the pilot plant tanks, occurring predominantly after shellfish manipulations

TABLE 14. Tapes COMPARATIVE GROWTH STUDY —PILOT PLANT VS. FLUME—INDIVIDUAL CLAM SIZE AND WEIGHT AND TOTAL POPULATION WEIGHT

	FLUME			PILOT PLANT		
BATCH NUMBER:	20	21	22	20	21	22
SIZE CLAM (length, mm)	20.9	22.1	21.4	25.0	26.0	23.5
WT CLAM (g)	1.7	1.9	1.6	3.4	3.7	2.7
TOTAL POP. WT (kg)	2.6	3.8	3.4	22.8	21.1	23.4

TABLE 15. Tapes COMPARATIVE GROWTH STUDY —PILOT PLANT VS. FLUME—VOLUME OF FOOD AVAILABLE PER SEC PER CLAM

	FLUME			PILOT PLANT		
BATCH NUMBER:	20	21	22	20	21	22
TOTAL POP. WT (g)	2600	3800	3400	22800	21100	23400
NO. CLAMS	2403	2094	2753	8233	8222	8586
FLOW (ml/sec)	18	18	18	440	440	440
FLOW (ml/sec/clam)	.01	.01	.01	.05	.05	.05

TABLE 16. Tapes COMPARATIVE GROWTH STUDY —PILOT PLANT VS. FLUME—MORTALITY TO-DATE (EARLY MAY 77)

	FLUME			PILOT PLANT		
BATCH NUMBER:	20	21	22	20	21	22
TOTAL MORTALITY (# CLAMS)	120	74	211	1767	1778	1414
% MORTALITY	5	3	7	18	18	14

and flow rate changes. No spawning has been observed in the flume populations.

It is evident from the collected data and daily observations that the pilot plant shellfish do not utilize their food supply efficiently. Growth rates of pilot plant shellfish have not equalled the "expected" growth rates, and flow rates have not been adjusted to the observed growth rates. Heavy concentrations of pseudofeces are observed.

3.2.7 Tapes Optimum Feeding Rate Experiment

This study has been designed to determine the optimum feeding and growth rates for various sizes of shellfish and to test the effects of a once-through flow in contrast to the present pilot-plant recirculation design.

Five different weight groups of clams are being used, calculated on the basis of individual clam weight and flow rate. The clams used in this experiment were selected from Tapes Batch #24's hatchery population.

The experiment is being conducted in clear acrylic tubes held vertically in a rack. Individual tube flow rates

are set at an initial rate of 2 ml/sec, controlled by metering pumps. Duration of each experimental run is tentatively four weeks. After review of data collected through Day 21, the experiment may be terminated at four weeks, or continued for a total of eight weeks. The size classes of clams are 10, 20, and 30-mm shell length.

Fifty percent of the experimental population will be returned to the hatchery flume at Day 28, and the other half will be used for growth measurements and meat protein analysis. Oxygen measurements are made at seven-day intervals with an oxygen meter (YSI Model 51A). Food for the clams (STX-167) is being supplied by reactor cultures. Protein samples on inflow and outflow are monitored three times per week. Clam movement and size distribution in the tubes are also monitored.

A parallel experiment is being run in the shellfish rack to reconfirm clam growth rates obtained in the Nov-Dec 1975 36-day constant-weight study (Roels et al., in press). Experimental differences are: monocultures instead of mixed culture diets, and no enrichment (trace metals + vitamins) vs. previously enriched cultures.

3.2.8 Preliminary Tapes Oxygen Experiment

A preliminary oxygen experiment was conducted to determine the effects of reduced oxygen concentrations on the survival and growth of shellfish in a once-through system.

Oxygen concentrations were monitored with an oxygen meter (YSI Model 51A) at 0800 and 1600 hrs daily in the preliminary pilot plant tank, with once-through flow but without aeration, and in the pilot plant recirculation tanks. One day per week, oxygen concentrations were monitored at four-hour intervals to establish a 24-hr oxygen cycle.

Growth rate measurements, population weight, individual clam weight and length determinations were made weekly on the preliminary pilot plant shellfish. Experiment duration was three weeks.

The shellfish in the preliminary pilot plant (once-through flow without aeration) had an increase in population weight of 40 g (2375.1 g - 2415.1 g). Mean individual clam weight and length increases were .3 g and .3 mm for the three-week period.

Growth measurements of preliminary pilot plant (PPP) shellfish are not directly comparable to pilot plant (PP) shellfish because of clam size and flow rate differences. The clams used in the PPP ranged from 29.5 - 35.9 mm; the PP clams were much smaller and the actual size range was greater (<4.0 - 18.0 mm). Flow rates were calculated in the PPP on the basis of clam weight gain per week. Because of the slow growth of the larger clams, the flow rate averaged 200 ml/10 sec. PP flow rates were calculated on the basis of extrapolated data from a Nov-Dec 1975 Tapes feeding study from 12-mm clams. Hypothetical flow rates

were set in the PP on calculated shellfish growth rates and not on observed rates. PP flow rates were considerably greater (180 ml/sec) than PPP flow rates.

Mortality of the PPP shellfish during the three-week period was 1%.

Dissolved oxygen and temperature readings were recorded daily at 0800 and 1600 hrs in the PPP and PP tanks, both in the inflow and outflow compartments. The dissolved oxygen concentration is maintained fairly evenly between 6 and 7 ppm (≈93-100% saturation) throughout the PP tanks by the recirculation system. Dissolved oxygen in the inflow compartment of the PPP tank averaged 91-96% saturation; however, after passing through the shellfish, it was 71-78% saturated in the outflow compartment. Approximately 20% of the dissolved oxygen appears to be removed by shellfish metabolism.

The 24-hr oxygen cycle (determined at 4-hr intervals) in the PPP and PP shellfish tanks was calculated. Both temperature and dissolved oxygen were recorded in the inflow and outflow tank compartments. The same pattern of high oxygen concentration is maintained throughout the recirculation tanks during the 24-hr period. The recirculation system appears to be functioning at approximately 90% efficiency.

Saturation values of oxygen during the 24-hr period were also determined. Saturation values peaked (100% or greater) in the PPP and PP at 1600 hrs and decreased sharply

at 2000 hrs. A maximum decrease in inflow vs. outflow oxygen saturation values of 28% occurred in the PPP tank at 2000 hrs. At no time did oxygen saturation values fall below 80% in the PP tanks and 70% in the PPP tanks. The lowest oxygen saturation values occurred between 2000 and 0400 hrs, in both the PPP and PP tanks.

Direct comparison of oxygen concentrations of once-through vs. recirculation flow cannot be made due to flow rate and clam population differences.

3.2.9 Effects of Recirculation vs. Once-Through Flow, and Weekly vs. Biweekly Cleanings, on Mortality of Tapes

To determine the effects of recirculation vs. once-through flow and weekly vs. biweekly cleaning on the PP shellfish mortality, the following changes were incorporated into the pilot plant system.

(a) Half of the PP tanks were changed to biweekly instead of weekly cleanings on March 8, 1977.

(b) On April 5, 1977, half of the PP tanks were converted to a once-through flow by turning off the aeration and stoppering the external airlift recirculation ports.

Mortality was recorded when the tanks were cleaned. The tank flow and cleaning arrangement is shown in Table 17. Mortality in the PP tanks on a recirculation flow (cleaned weekly) prior to this study is given in Table 18. Five-week mortality figures from weekly and biweekly cleaning are shown in Table 19 for the recirculation system.

TABLE 17. SHELLFISH TANK FLOW AND CLEANING ARRANGEMENT

TREATMENT:	ONCE-THROUGH weekly	RECIRCULATION weekly	ONCE-THROUGH biweekly	RECIRCULATION biweekly
TANKS :	1,6,9	2,5,10	4,7,12	3,8,11

TABLE 18. SHELLFISH MORTALITY IN TANKS ON A RECIRCULATION FLOW SYSTEM AND CLEANED WEEKLY

DATE	MORTALITIES IN TRAY NUMBER 1	2	3	4	5	6	7	8	9	10	11	12
01/11/77	-								-			<10
01/18/77	-								-			<12
01/25/77	38				15				57			
02/01/77	46	14	40	22	14				50			
02/08/77	30	14	21	14	26				35			
02/15/77	9	13	18	20	23				63			
02/22/77	17	28	114*	19	3	4	6	4	39			
02/24/77	9	13	9	6	-	-	-	-	-			
03/01/77	7	6	23	18	6	12	1	5	4	2	12	3
TOTAL:	156	88	225	99	87	16	7	9	248	2	12	25

*tray accidentally dropped

TABLE 19. FIVE-WEEK SHELLFISH MORTALITY FIGURES ON A RECIRCULATION FLOW SYSTEM AND CLEANED WEEKLY AND BIWEEKLY

DATE	MORTALITIES IN TRAY NUMBER											
	1	2	5	6	9	10	3	4	7	8	11	12
	WEEKLY CLEANING						BIWEEKLY CLEANING					
03/08/77	31	28	12	10	16	8	26	35	-	-	9	18
03/15/77	17	25	132	14	25	16	-	-	27	11	-	-
03/22/77	36	33	18	35	28	25	67	40	-	-	37	41
03/29/77	37	34	231	25	48	33	-	-	38	23	-	-
03/31/77			430*									
04/05/77	52	31	295	22	53	25	89	70	-	-	108	123
TOTALS:	173	151	1118	106	170	107	182	145	65	34	154	182

*Antibiotic treatment (Streptomycin sulfate).

Mortality was particularly severe in tray #5 to which antibiotic was added (.25 mg/l of Streptomycin sulfate). Samples of the clams were sent to the Middle Atlantic Coastal Fisheries Center, Pathobiology Laboratory, Oxford, Maryland, for analysis.

Heavy fecal accumulations were observed under and along the edge of the shellfish trays and some evidence of fecal decomposition was present. Shellfish tray-liners became clogged with fecal material and pseudofeces, thus decreasing flow circulation and causing "dead" areas in the tank. Accumulation of organic matter was also observed inside the recirculation plumbing and in the effluent drain.

The mortality of shellfish in tanks cleaned weekly and biweekly on both recirculation and once-through flow systems is shown in Table 20.

Total mortality for Batch #20, 21, and 22 is 1767, 1778, and 1414, respectively, or 18, 18, and 14%.

3.2.10 Macroalgae

The first half of the period covered by this report dealt with parameters of nitrate and ammonia uptake by Hypnea musciformis on the beach and in the laboratory. Laboratory experiments were run using the Technicon AA-II to determine constant NO_3/NH_4 uptake rates for H. musciformis and Macrocystis pyrifera. The conclusions will be presented in the final report to the agency.

TABLE 20. SHELLFISH MORTALITY IN TANKS ON A RECIRCULATION FLOW AND ON A ONCE-THROUGH FLOW AND CLEANED WEEKLY AND BIWEEKLY

TREATMENT:	ONCE-THROUGH weekly			RECIRCULATION weekly			ONCE-THROUGH biweekly			RECIRCULATION biweekly		
TANKS :	1	6	9	2	5	10	4	7	12	3	8	11
04/12/77 :	50	9	47	29	9	19	--	70	--	--	26	--
04/19/77 :	53	29	60	30	5	23	78	--	82	61	--	69
04/26/77 :	52	22	63	50	6	18	--	96	--	--	41	--
05/03/77 :	16	18	20	34	5	19	55	--	51	40	--	75
TOTALS :	171	78	190	143	25	79	133	166	133	101	67	144

The second half of the present period was devoted to continuous culture of H. musciformis in 200-gal containers on the beach. We have, in this period, identified several problems associated with culture of this type. The growth rates obtained in beach cultures of H. musciformis were abnormally low for the months of February and March (3.3-3.6% per day, as compared to 8.4, 10.3 and 15.8% per day for the same period last year). It was noted that previous studies had differed only in the fact that the source of shellfish effluent is microalgae grown in 100% unsupplemented deep water. In previous studies, shellfish were fed partly on microalgae grown in deep water supplemented with chelated trace metals and vitamins. Experiments are now being done in the laboratory and beach cultures to determine which factors limit Hypnea growth in our system. Data from this experiment indicate that trace metals and/or vitamins are indeed a major limiting factor. For the first twenty days, growth rates for tanks enriched to the level of 1.6 µm Fe showed 10.7% growth/day over a 20-day period while unenriched seaweed grew 4.1%/day over the same period.

Laboratory experiments are now in progress to determine exact requirements of Hypnea for all common trace metals and B-vitamins.

Experiments to compare carrageenan production of saprophytic and gametophytic phases of H. musciformis were

abandoned due to insufficient control of the factors which affect fertility. Unwanted sporulation and resultant mixture of haploid and diploid plants makes cultures useless for the experiment described.

During the period of slow growth in beach cultures, infestation with epiphytes became critical. However, in the present study, where 10.7% growth/day occurs, no epiphytes have been observed. It seems that rapidly growing H. musciformis competes favorably with chlorophyte epiphytes.

Complete data on all experiments briefly described above will be presented in the final report to the agency.

3.2.11 Hatchery

The primary function of the hatchery from July 1976 through the present was to supply populations of Tapes japonica, on a regular basis, to the pilot plant and experimental shellfish areas. Fourteen batches of Tapes were reared in the hatchery and all but one of these batches successfully completed metamorphosis (Table 21). Batches #20, 21, and 22 were used in the pilot plant.

One striking difference between batches #21 & 23 is the percent survival to metamorphosis. Survival appears to be decreasing, from the average of 40% in Batches #5-13 (Table 22), with successive batches of Tapes. The average survival to metamorphosis for Tapes Batches #16, 17, 18 was 51% (Table 23).

There are several factors which could account for

TABLE 21. *Tapes japonica* REARED IN THE HATCHERY FROM AUGUST 1976 THROUGH MAY 1977

SPAWNING DATE	BATCH #	INDUCED/SPONTANEOUS	% SURVIVAL TO METAMORPHOSIS
08/03/76	20	induced	33%
08/16/76	21	induced	14
08/24/76	22	induced	10
08/31/76	23	induced	11
01/03/77	24	spontaneous	6
01/24/77	25	spontaneous	4.5
01/31/77	26	spontaneous	0.4
02/08/77	27	spontaneous	15
02/28/77	28	induced	2
03/15/77	29	spontaneous	0
03/??/77	30	?	40
03/30/77	31	spontaneous	6
04/01/77	32	spontaneous	13
04/25/77	33	induced	yet unknown

TABLE 22. *Tapes japonica* LARVAE BATCHES FROM FEBRUARY 1975 TO JANUARY 1976

SPAWNING DATE	BATCH #	% SURVIVAL TO METAMORPHOSIS
02/04/75	5	41
03/05/75	6	44
04/05/75	7	63
07/16/75	9	34
10/16/75	10	36
12/30/75	11	4
01/02/76	12	51
01/27/76	13	46

TABLE 23. *Tapes japonica* REARED IN THE HATCHERY IN MAY AND JUNE 1976

SPAWNING DATE	BATCH #	INDUCED / SPONTANEOUS	% SURVIVAL TO METAMORPHOSIS
05/12/76	16	induced	60
06/02/76	17	spontaneous	30
06/18/76	18	induced	63

this decreased survival in the batches of Tapes grown from August 1976 through the Spring 1977.

(a) The single deep-water pipe in the hatchery has never been cleaned since operation began in December 1974. At times, H_2S is present in the hatchery plumbing when the deep water is first turned on. To reduce the H_2S, a valve is now left partially open to allow deep water to flow at all times.

(b) Since the hatchery operation began, the antibiotic Streptomycin sulfate ("Vet Strep") has been used in the larval cultures at 50 ml/liter. Bacteria do build up a resistance to this antibiotic, therefore others are being tested.

(c) A change in hatchery personnel as of August 1976 may also have had an influence of survival of hatchery-reared Tapes.

Suggestions for eliminating any present problems and for helping to increase survival in the rearing of Tapes larvae are the following:

(a) Installation of a second deep-water piping system in the hatchery. It is proposed to alternate the use of one set of pipes one week with the other set of pipes the next week.

(b) Installation of inspection sleeves or unions at various intervals along the present deep-water line to aid in cleaning (chloroxing or acid-washing) or air-drying when

the line is not in use.

The first proposal (a) is preferable since the weekly labor involved, once the second piping system is installed, would be less. Small-scale studies to be started in June 1977 will test the possibility of deep-water hatchery-line contamination as a cause of decreased survival.

(c) Testing additional antibiotics: Sulfamethazine -"Vimethazine"; Terramycin (Oxytetracycline) and Neomycin Sulfate. It is possible that with a second deep-water piping system a lower concentration of antibiotics (or no antibiotics) would be needed for the hatchery operation.

Since the first batch of Tapes japonica larvae was reared in the "Artificial Upwelling" mariculture system in April 1974, Tapes have been induced to spawn, by thermal and chemical (stripped gonads) stimulation, nine months of the year (Table 24); spontaneous spawnings have been observed seven months of the year. Generally, Tapes larvae have been obtained whenever a batch was required.

After rearing 33 batches of Tapes in the hatchery system, it is apparent that the feasibility of a hatchery in the tropics has been demonstrated. Of course, refinements and improvements in the system are necessary.

In the renewal application we will discuss in more detail suggested procedures for increasing the efficiency and decreasing labor and equipment costs on the present hatchery operation.

TABLE 24. *Tapes japonica* REARED IN THE HATCHERY APRIL 1977 THROUGH MAY 1977

MONTH	# BATCHES INDUCED TO SPAWN	# BATCHES SPAWNED SPONTANEOUSLY
JAN	2	3
FEB	1	2
MAR	2	2
APR	2	3
MAY	2	-
JUN	-	2
JUL	3	-
AUG	4	-
SEP	-	1
OCT	1	-
NOV	-	-
DEC	1	1

4. AQUACULTURE BUDGET GENERATOR

The budget generator computer program, adapted by Dr. Geoffrey P. Allen from a similar program used at the University of California to investigate the economics of lobster aquaculture, has been verified and used to explore a set of operating conditions for the "Artificial Upwelling" pilot demonstration plant.*

The language in which this program is written is Extended ALGOL, which restricts its use to compatible installations such as the Computer Center of the University of California, Davis.

4.1 Program Listing

A recent listing of the budget generator computer program is included with this report under Appendix E.

4.2 Program Updating

No extensive updating has been undertaken, pending a translation of the program and its transfer to the University of Texas at Austin computer system. The program is used in its present form, with the following restrictions or corrections:

- VAR[27] = COEFFICIENT b_2 IN THE EXPRESSION FOR η_1 (PHYTOPLANKTON) IS TO BE CHANGED

  FROM: -1.750×10^9

  TO: -1.340×10^9

*A report on the genesis of this program was presented as Appendix II to our 1976-1977 Renewal Proposal.

- VAR[24] = UNIT FLOW RATE INTO PHYTOPLANKTON TANKS

FROM: 0.00106 $cm^3/cm^2/sec$

TO: 0.001331 $cm^3/cm^2/sec$

- Single batch mode with VAR[1] = 0, is not correct.

An illustration of the application of this program, showing the influence of pool depth and turnover rate on the production cost, is included as Appendix F. Figure 8 presents the computed relationship of cost versus amount of deep-sea water handled, per day, for different pool depths.

An examination of individual printouts reveals the major cost items, which are, at present:

(a) Recirculation pumping in the shellfish trays.

(b) Phytoplankton space and recirculation costs.

Remark: Deep-sea water has been assumed available, free; only the energy required to move it through the system is included. This assumption is based on a combined energy/mariculture operation.

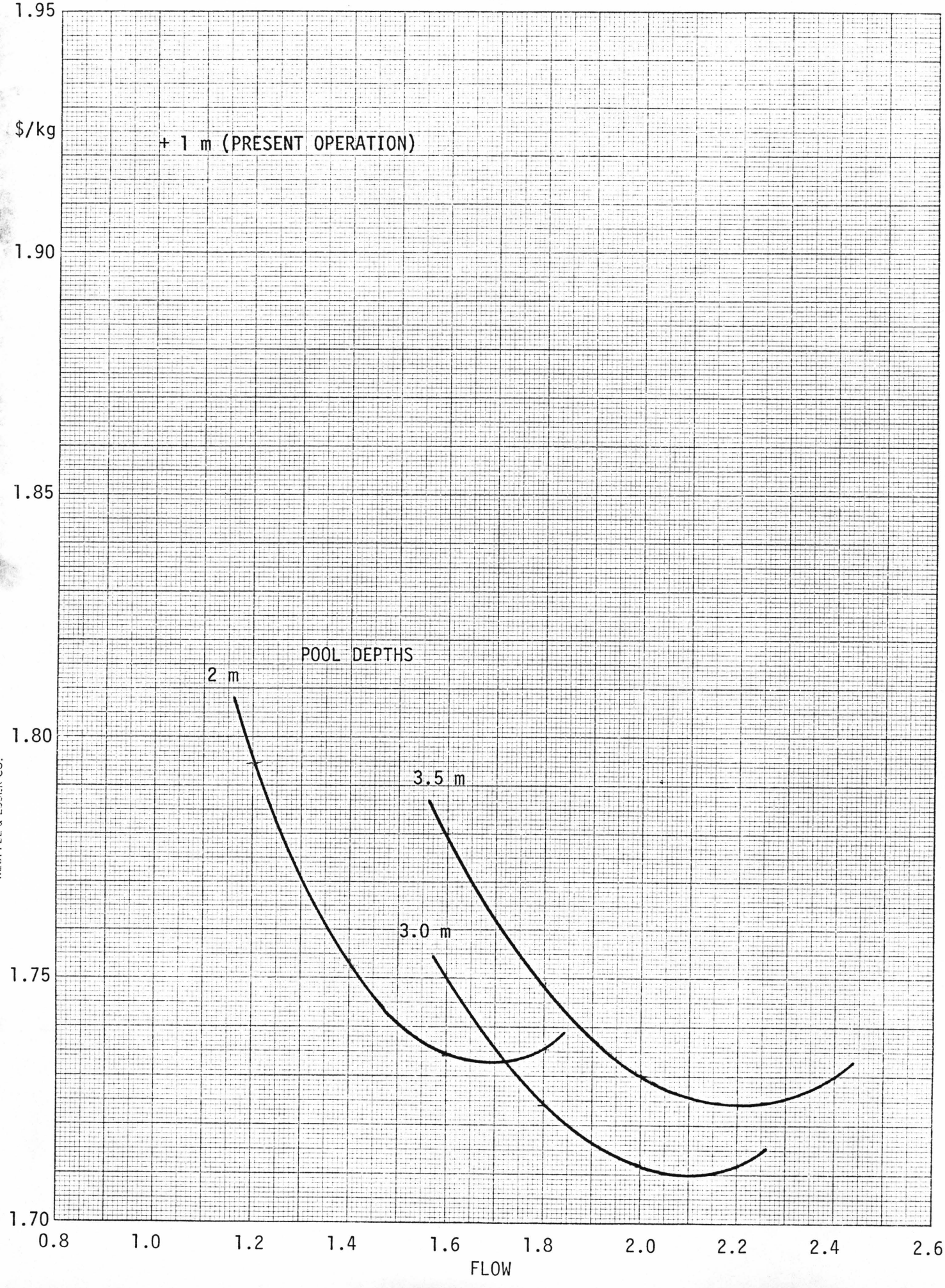
During the month of March, Dr. Allen undertook a revision of the program, in order to:

(a) incorporate permanently the corrections noted above;

(b) obtain a printout of manpower requirements;

(c) correct the expression used for illumination when the pool depth exceeds the critical depth;

Figure 8. Computed relationship of cost ($/kg) vs. amount of deep-sea water handled (flow), per day, for different pool depths.



KE 10 X 10 TO THE CENTIMETER 46 1521
18 X 25 CM. MADE IN U.S.A.
KEUFFEL & ESSER CO.

(d) remove the sections of the program which are inactive, in our application, to simplify translation of the program.

Dr. Allen provided us with a list of recommended changes which have not yet been introduced into the program. He also re-examined and documented the source for individual cost factors used in this program.

A copy of his report is included as Appendix G.

5. PAPERS PRESENTED AND/OR PUBLISHED

(d) remove the sections of the program which are inactive, in our application, to simplify translation of the program.

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5. PAPERS PRESENTED AND/OR PUBLISHED

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APPENDIX A

ARTIFICIAL UPWELLING MARICULTURE:

TECHNICAL DESCRIPTION

May 1977

ARTIFICIAL UPWELLING MARICULTURE

TECHNICAL DESCRIPTION

Introduction

— Organic material is synthesized in the ocean from inorganic compounds. This transformation is performed by autotrophic organisms utilizing sunlight as a source of energy. The most important photo-autotrophs, quantitatively, are the algae (1; p. 58).

— Both energy and inorganic material (nutrients) are required to sustain the production of high-energy organic compounds. In tropical oceans, the "primary productivity" is limited by the rate of nutrient renewal. Much of these nutrients is derived from the decomposition of organic matter. Whenever this decomposition occurs below the euphotic zone, the nutrients remain unused and, except in areas of natural upwelling, return to the surface by slow diffusion. High nutrient concentrations are encountered at depths of 500 fm. (900 m)(2; p. I-19). This nutrient-rich deep-sea water can be brought to the surface with a minimal expenditure of energy.

— Direct harvesting of planktonic algae (phytoplankton) is impractical because of the small size and low concentration at which these organisms occur. The phytoplankton can sustain larger animals, which constitute usable sources of human food.

— A set of conditions under which a two trophic-level phytoplankton-shellfish "artificial upwelling" mariculture can operate has been established (3). Extrapolation of the collected data to large installations, and the economic optimization of such plants, requires a definition of the laws governing total productivity.

Objective

Our objective is to establish an adequate quantitative relationship (transfer function) between the mariculture's outputs, its inputs, and its environment.

The expressions are to be based on our understanding, or best interpretation, of the mechanisms or reactions involved. They will be deemed adequate if their application yields predictions within 10% of actual observations, or within observational error, if larger.

In areas where no logical mechanism can be introduced, empirical expressions satisfying experimental evidence may be used. These sections are identified in the margin and are subject to a continuing analysis.

Initial Hypothesis

I. A spectrum of elements is involved in the exchanges taking place within our system. "Nitrogen" is considered the limiting chemical component in the food stream.

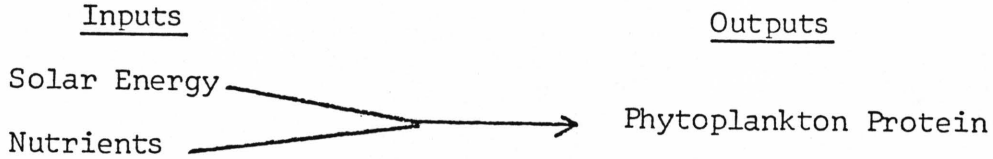
II. Each trophic level is considered an independent entity, related to the other level only through the output/input link, as defined.

III. Some operating conditions can be controlled and/or

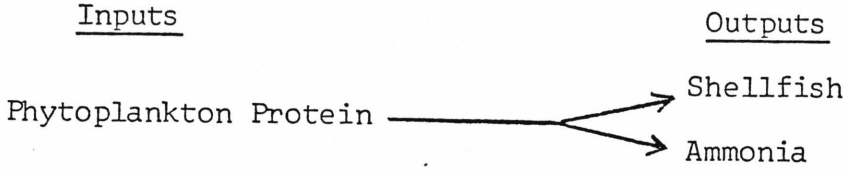
maintained constant. Others have time-dependent fluctuations. It is assumed that storage mechanisms within the organisms will absorb these fluctuations and allow time-variant inputs to be replaced by constant averages.

Definition of Trophic Levels

- First Level : Phytoplankton



- Second Level : Shellfish



First Trophic Level : Phytoplankton

Phytoplankton absorbs nutrients and transforms them into protein with the help of solar energy, according to:

$$P = c \alpha \eta_1 v$$

where:

P	= phytoplankton protein produced	(g)
c	= nutrient concentration	(g-at. N cm^{-3})
α	= equiv. N to protein ratio (14 x 6.25) = 87.5	-
η_1	= conversion efficiency of NO_3-N to phytoplankton protein-N	-
V	= volume of deep-sea water handled	(cm^3)

η_1, for a specific phytoplankter, is a function of the exposure to sunlight of the culture. This exposure is, in turn, a function of the pool turnover rate and of the pool depth.

At a compensation depth (D_c) the light intensity equals the respiration needs of the phytoplankton (1; p. 83).

If this depth is exceeded, a net decrease in total production results.

The compensation depth corresponds approximately to the depth at which incident light is attenuated by a factor of 0.01. At that depth:

$$e^{-\mu D_c} = 0.01 \quad \therefore$$

$$-\mu D_c = \ln 0.01 = -4.61$$

where μ = extinction coefficient (cm^{-1})

The average light attenuation, in a column of depth D_c is:

$$\frac{I_{av}}{I_o} = \frac{1}{D_c} \int_o^{D_c} e^{-\mu z} dz = \frac{1}{\mu D_c} (1-e^{-\mu Dc}) = .215$$

where z is the vertical axis.

From experimental data collected in St. Croix by Mary W. Farmer (4), a reactor shaded to 20% of incident light could be operated at a turnover rate of .7 day^{-1}. The measured value of μ = .01158 cm^{-1}, and:

$$D_c = \frac{\ln 0.01}{-\mu} \approx 400 \text{ cm}$$

In reactors approximately 80 to 100 cm deep, a turnover rate of 1.2 day^{-1} can be maintained.

A linear expression for turnover rate versus depth can be derived to fit both data points. For:

$$\text{turnover rate} = f(z) = k_i + az = \frac{\dot{V}}{Az} \qquad (sec^{-1})$$

where: $\dot{V}$ = deep-sea water flow rate $(cm^3\ sec^{-1})$

A = pool area (cm^2)

$$f(100) = 1.2/(24 \cdot 3600) = 13.9 \times 10^{-6}$$

$$f(400) = 0.7/(24 \cdot 3600) = 8.10 \times 10^{-6}$$

$$a = \frac{(8.10-13.9)\times 10^{-6}}{400-100} = -1.93 \times 10^{-8}$$

$$k_i = (13.9\times 10^{-6}) - 100\ a = 13.5 \times 10^{-6} \qquad (sec^{-1})$$

The turnover rate expression can be normalized into:

$$\frac{\dot{V}}{Az} = k_i\left[1 + \left(\frac{aD_c}{k_i}\right)\frac{z}{D_c}\right]$$

and rounded:

$$\approx k_i\left(1-\frac{z}{2D_c}\right)$$

Note: The value of D_c, as determined from MWF's experiment, may be different for different phytoplankters. If a higher concentration of nutrients is used (c), the cultures are expected to have a higher density and extinction coefficient. The product μD_c should remain constant, which will be approximated by maintaining $c \cdot D_c$ constant (neglecting the contribution to μ of clear seawater).

In the expression which relates the turnover to the pool depth, $^1/k$ represents the exposure factor.

η_1 can now be defined as a function of k. In our present pool system, with a 1-m depth and a turnover rate of 1.15 day^{-1}

$$\frac{\dot{V}}{Az} = 1.33 \times 10^{-5} = k\left(1-\frac{z}{2D_c}\right)$$

and: $k = 1.52 \times 10^{-5}$

Measured conversion efficiency (5), averaged over a 36-day period, under these operating conditions, shows that:

$$\eta_1 = .69$$

No other measurements allowing us to relate η_1 to k are available at this time.

It is known, however, that high turnover rates lead to a "washout" of the culture, resulting in a drastic drop in conversion. It is also impossible for η_1 to exceed 1.0 even at high values of the exposure factor.

Evaluating the coefficients of:

$$\eta_1 = a_2k^2 + a_1k + a_0$$

for:

$$\begin{cases} \eta_1\big|_{k=0} = 1 \\ \dfrac{d\eta_1}{dk}\Big|_{k=0} = 0 \\ \eta_1\big|_{k=1.52\times10^{-5}} = .69 \end{cases}$$

yields:

$$a_0 = 1$$

$$a_1 = 0$$

$$a_2 = -1.34 \times 10^9$$

$$\eta_1 = 0 \text{ for } k \sqrt{\frac{1}{1.34\times10^9}} = 2.73 \times 10^{-5}$$

This value of k corresponds to a turnover rate of 2.06 day^{-1} in a 1-m pool, or 1.18 day^{-1} in a 4-m pool (or .215 shaded reactor). At these turnover rates, complete "washout" can be expected.

Second Trophic Level : Shellfish

Shellfish assimilates phytoplankton, and in the process increases its wet weight, according to:

$$S = \beta \eta_2 P$$

where:

S = shellfish wet weight increase (g)

β = protein to wet weight ratio (33.125) -

η_2 = conversion efficiency of plant protein to animal protein -

η_2, for a specific phytoplankton/shellfish combination, is a function of the rate at which phytoplankton is presented to the shellfish. If this rate is low, only the vital needs will be

satisfied, and no net weight increase will result ($\eta_2 = 0$). If the rate is too high, all available food cannot be assimilated.

Growth rates, in terms of $\frac{\dot{w}}{w}$, are not constant, over the life span of the shellfish. Studies have shown that over a wide range of sizes, the filtration rate of shellfish is proportional to $w^{0.73}$, where w represents the individual weight of each animal (6). A further decrease of this rate, for large (or old?) animals was noticed, but not evaluated.

The filtration rate, and the criterion by which to characterize feeding rate, seems more closely related to the animal's area $\left[l^2=(\frac{w}{\rho})^{\frac{2}{3}}\right]$ than to its weight, or volume ($l^3= \frac{w}{\rho}$).

In our analysis we have adopted:

$$F = \frac{\dot{P}}{N(w)^{\frac{2}{3}}} \qquad (g^{\frac{1}{3}}\ sec^{-1})$$

as our feeding criterion.

F = protein feeding rate per (individual weight)$^{\frac{2}{3}}$

$\dot{P}$ = phytoplankton protein inflow rate ($g\ sec^{-1}$)

N = number of animals -

w individual weight (g)

Applying this criterion to data collected in St. Croix during a constant-weight study in November-December 1975, has provided the following quadratic least-square fit expression for $\eta_2 = f(F)$:

$$\eta_2 = a_2(\ln F)^2 + a_1(\ln F) + a_0$$

$a_0 = -16.89144976$

$a_1 = -\ 1.891173337$

$a_2 = -\ 0.0518$

within the following boundary:

$$7 \times 10^{-9} \leq F \leq 9 \times 10^{-8}$$

Notes & References

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APPENDIX B

NITROGEN BALANCE AND CLAM GROWTH IN AN ARTIFICIAL UPWELLING MARICULTURE SYSTEM AT DIFFERENT FOOD FLOW RATES AND SHELLFISH DENSITIES

by

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Running head: Nitrogen balance and clam growth.

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Abstract

The conversion of deep-sea water nitrate to algal protein and further to clam-meat protein was studied at the St. Croix Artificial Upwelling Project (U.S. Virgin Islands), where nutrient-rich water, pumped from 870-m depth in the sea, is used as the raw material for a mariculture system. Unialgal cultures of Chaetoceros curvisetus (STX-167) and S-1, an unidentified naked flagellate, were grown individually and continuously in onshore pools provided with deep-sea water. The cultures were combined 1:1 in a mixing tank and fed continuously to several batches of Tapes japonica for 36 days. Thirty-five, 70 and 140 g batches of clams, in 4-liter containers, received a continuous food flow-rate of 1 ml/sec. Thirty-five, 50, 70, 100, and 140 g batches of clams, in 4-liter containers, received a 2 ml/sec food flow-rate. The particulate protein nitrogen and dissolved NH_4^+ and (NO_3^- + NO_2^-) entering and leaving each shellfish tank were measured daily. Every nine days all of the clams were weighed and measured, enough clams were harvested from each batch to bring the total weight back to its starting level, and the tank deposit was separated, measured and analyzed for each group.

Sixty-nine percent of the deep-water nitrate-nitrogen (concentration 31 µg-at/l) was converted into algal protein-nitrogen over the 36-day period. From 31% to 35% of the algal protein entering the Tapes feeding tanks was converted into clam meat protein by the 1 ml/sec flow groups, and between 24% and 33% of the algal protein was converted into clam meat protein by the 2 ml/sec flow groups.

Maximal individual clam growth was obtained in the 35 g, 2 ml/sec group, with a 1.42 mm/week shell-length increase and a 41.1% weekly increase in fresh weight per clam. This fastest individual clam growth was obtained at the lowest percent stripping of algal protein. The greatest clam population growth occurred for the 100 g, 2 ml/sec group, with a total weight gain of 134 g in 36 days.

Ammonium ion concentration increase in the shellfish tank was highest at the slowest individual clam growth.

The Protein Efficiency Ratios in this experiment varied between 8 and 14, indicating that the algal food source used is a good one for Tapes japonica.

Introduction

In our Artificial Upwelling Project in St. Croix, Antarctic Intermediate Water is pumped from 870-m depth in the sea into 45,000-liter concrete pools on shore in which unialgal cultures of planktonic diatoms are grown continuously. The pool cultures are started by inoculating them with cultures grown in 757-liter polyethylene tanks. The growth rate of the algae is regulated by the rate at which nutrients are supplied by the incoming deep water; the deep water flow is regulated to assure nearly complete utilization of the nutrients in the deep water.

The system produces 114,000 l of nearly unialgal diatom culture per day (10^4-10^6 cells/ml) which is pumped continuously into shellfish tanks at metered rates based on the feeding activity of the animals. The total flow pumped to the shellfish matches the flow of deep water into the algal pools, so that the pool volume remains constant. The filter-feeding shellfish remove up to 90% of the algae pumped from the pools. The yearly temperature range in the shellfish tanks is 22-29°C.

Ten species of shellfish have been screened for growth and survival in the St. Croix system. Seven species grew well and reached market size quickly. They are: Ostrea edulis, european oyster; Crassostrea gigas, pacific oyster; C. gigas, Kumamoto variety; Tapes japonica, japanese little-neck clam; Mercenaria campechiensis, southern clam or quahog; F_1 clam, a cross between M. campechiensis x M. mercenaria; Argopecten irradians, bay scallop. Pinctada martensii, the pearl oyster, is also growing very rapidly in the system.

Panulirus argus, the spiny lobster, Strombus gigas, the queen conch and carrageenin-producing seaweeds are grown in the effluent of the shellfish tanks (Othmer and Roels, 1973; Roels et al., 1976).

The purpose of the present experiment was to determine

1. optimum shellfish densities and food flow rates to achieve maximum shellfish growth
2. the efficiency of "nitrogen"-transfer in this food chain, from deepwater nitrate to phytoplankton protein, to clam meat protein.

In the present experiment, unialgal cultures of the diatom Chaetoceros curvisetus (St. Croix 167) and of S_1, an unidentified naked flagellate were grown in deep water as food for the clam, Tapes japonica.

Tapes japonica was chosen as a test animal because it grows well in the system and can easily be spawned in our St. Croix hatchery. We have demonstrated earlier that a mixture of Chaetoceros curvisetus (St. Croix 167) and S_1 is a good food for Tapes japonica in our system (Rodde et al., 1976).

Materials and Methods

Animals

The clams used in this nitrogen balance and growth experiment were Tapes japonica (Deshayes). The average length of the clams was 12.7 mm at the beginning of the study. Only the fastest growing clams comprising the top 25%, less the top 1%, of the original population were used for our work. These were second

generation clams grown at the Artificial Upwelling Mariculture Station on St. Croix from brood stock originally obtained from Pacific Mariculture in California. Standard spawning procedures outlined by Loosanoff and Davis (1963) were used.

Phytoplankton

The clams were fed a mixed diet of Chaetoceros curvisetus (STX 167) and S-1, an unidentified naked flagellate. The mixed algal diet contained on the average 21.5 µg-at PPN (particulate protein nitrogen) per liter. Previous work has shown the PPN of these phytoplankton to be 90% of the total nitrogen (Dorsey et al., 1977). Assuming a chemical composition equal to that given for Chaetoceros sp. by Parsons et al. (1961), the dry weight of this algal suspension would be approximately 5.7 mg/l. At the two flow rates employed in this experiment, 938 or 469 µg-at PPN day^{-1}, equivalent to 246 or 123 mg dry weight of algal cells, were fed per day to each batch of Tapes in a 4-liter tank. A cell suspension with 22 µg-at PPN contains 5.3×10^4 cells/ml $\pm$ 15% of STX 167 or 1.2×10^5 cells/ml $\pm$ 15% of S_1. Chaetoceros curvisetus (STX 167) was isolated by Dr. Kenneth Haines at the Artificial Upwelling Station and grows well at temperatures up to 30°C. The S-1 flagellate was obtained from Guillard and isolated by him from the Sargasso Sea.

Chemicals

All chemicals for the protein and inorganic assays were obtained from Fisher Scientific, Freehold, New Jersey, or from J. T. Baker Chemical Co., Phillipsburg, New Jersey. The crystallized and lyophilized bovine serum albumin protein

standard came from Sigma Chemical Co., St. Louis, Mo.

Experimental Design

Nutrient-rich deep sea water containing 31 µg-at liter^{-1} NO_3^- was pumped continuously through three 3" diameter polyethylene pipes from 870 meters below the sea surface into a 12,000 gallon concrete pool used for growing Chaetoceros curvisetus. S_1-was grown in continuous culture in several 2,000 liter concrete containers. The turnover rate for both cultures was 1.1/day. Backup cultures of the phytoplankton were always ready in case of mishap or contamination to maintain an average of 21.5 µg-at PPN/l for the mixed culture.

The cultures of Chaetoceros curvisetus and S-1 flowed at 23 ml/sec each into a 40-liter cylindrical polyethylene mixing container. The culture mix was fed to sixteen shellfish tank feeding lines at constant pressure. Figure 1 gives a schematic diagram of the experimental layout.

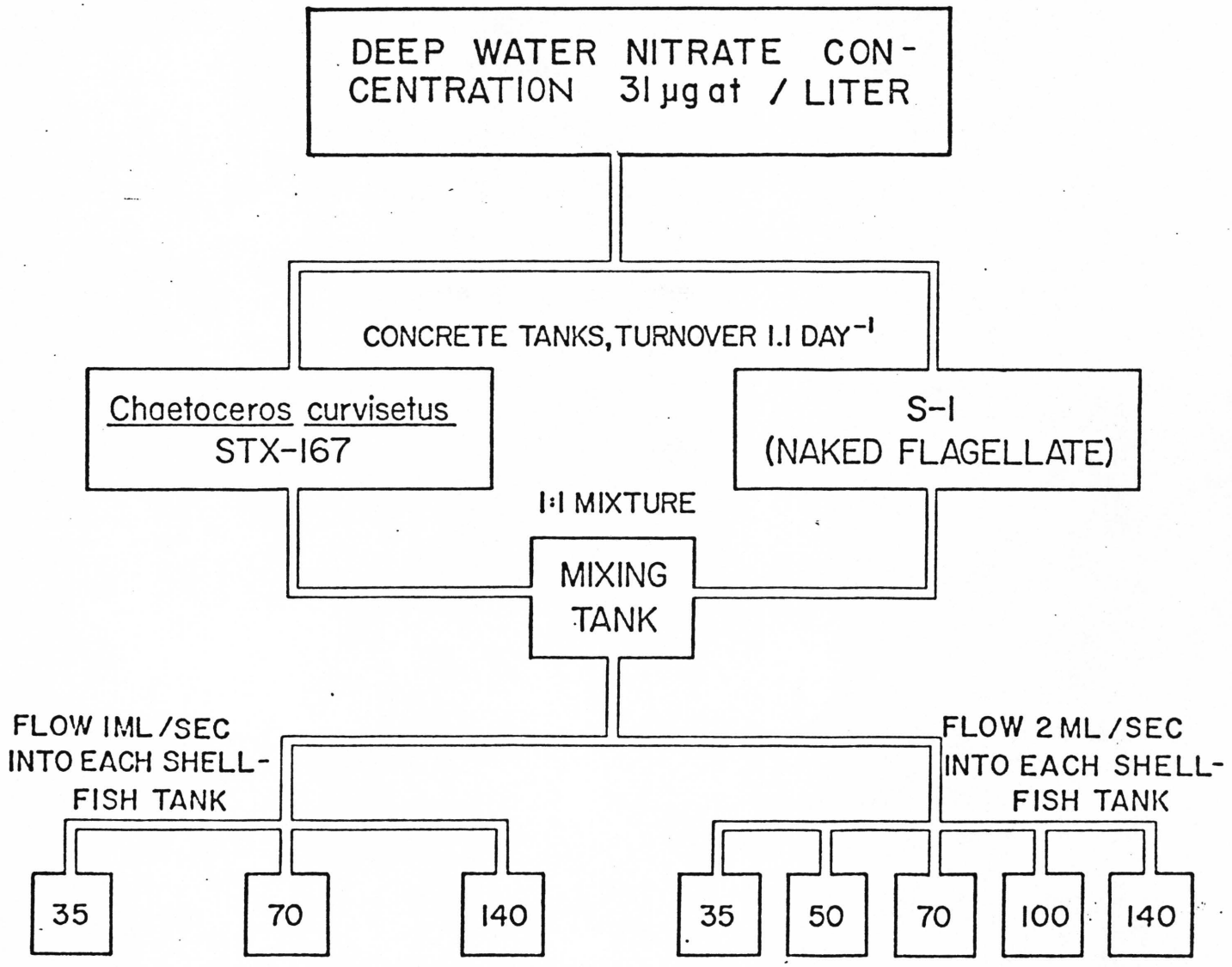
Figure 1

The mixing chamber was vigorously stirred by an air stream. The outflow from the mixing tank flowed to 16 shellfish tanks with a capacity of 4 l each.

The flow to the 4-liter shellfish tanks (12" x 7.75" x 5.13") was regulated to provide either 2 ml/sec or 1 ml/sec. The flow to each shellfish tank was controlled by a length of 1/16" teflon tubing coming from the feed level pipe.

The shellfish feeding tanks had a perforated air tube at each end to mix and aerate the water. In addition 1/4" chicken screening,

DIAGRAM OF NITROGEN BALANCE STUDY



coated with polyester resin to avoid metal ion contamination, was laid horizontally 1.5" off the tank bottom. This screening kept the clams off the bottom and prevented them from coming in contact with the tank deposit. No shellfish died during this experiment.

The shellfish tanks had an overflow tube keeping their volume at 4 liters. One set of shellfish tanks received an algal culture flow of 2 ml/sec and another set received 1 ml/sec resulting in turnover times of 33 or 66 minutes respectively. The 2 ml/sec flowrate provided 173 l/day and the 1 ml/sec flowrate provided 86.5 l/day or algal culture to the shellfish.

The clam densities used were 35, 70 and 140 g per 4.0 liter tank for the 1.0 ml/sec flow and 35, 50, 70, 100 and 140 g per 4.0 liter tank for the 2.0 ml/sec flow. All experiments were run in duplicate, i.e. there were 16 experimental shellfish tanks in operation simultaneously for 36 days.

The salinity of the system was 34.75-34.95% and the temperature was $25^{o}C \pm 3^{o}C$.

The flow rates into the algal culture tanks and out of the shellfish feeding tanks were measured and adjusted, if necessary, at 8 o'clock in the morning and again at 2 o'clock in the afternoon each day of the experiment. Flow rates were adjusted if they deviated by 2% or more from the planned rate.

Sampling

Duplicate one-liter samples of the mixing-tank outflow were taken each day at 2 o'clock for the determination of the particulate protein nitrogen and dissolved NO_3^-, NO_2^- and NH_4^+.

Duplicate two-liter samples of the shellfish-tank outflow were taken each day at 2 o'clock for the determination of particulate protein nitrogen and dissolved inorganic nitrogen leaving the shellfish feeding tanks for each of the different experimental treatments.

The test populations of Tapes japonica were weighed, counted, measured and culled back to the original clam weight on days 9, 18, 27 and 36 of the experiment. After all measurements were taken, the culled animals were frozen until we were ready to determine wet and dry meat weight and meat protein nitrogen.

For simplicity, the experimental treatment in which 35 g of Tapes were kept in a 4-liter tank receiving a flow rate of 1 ml/sec is referred to as the 35 g, 1 ml/sec group.

Analytical Methods

Clam growth. The clam population growth rate of Tapes japonica was measured by the increase in the mass of each batch of whole clams. The individual clam growth rate was determined by measuring the average increase in length of 25 culled clams at the end of the 36-day experiment, using a micrometer.

Whole clam weight and wet meat weight. Tapes japonica were blotted dry with paper towels and weighed alive for whole clam weight. To get the wet meat weight, the shellfish were first frozen and then shucked by using a spatula to open the clams and to scoop out the frozen meat as a single mass. The wet meat was then placed in a tared glass petri dish and weighed. This method of shucking was necessary because of the small size and the large number of clams used in this experiment. The frozen meat could

be quickly and more easily removed in this manner. While shucking a batch of clams, the remaining clams in that same batch were kept from melting by keeping them on a surface kept at low temperature.

Dry meat weight. Each batch of clam meat was freeze-dried at 60°C for 24-48 hr and then placed in a desiccator for several days. After the clam meat had reached constant weight, the dry meat was scraped from the glass petri dish completely with a single-edged razor blade and weighed on a glassine paper. The clam shells were dried separately in a similar manner.

Shell weight and shell protein. The wet and dry weights of the shells were determined. After the dry weights were taken, the shells were ground to a fine powder with a mortar and pestle. Ten mg of ground shell gave an appropriate final color absorbance with the heated biuret-Folin assay for protein-nitrogen measurement (Dorsey et al., 1977).

Clam protein nitrogen. The dry clam meats were pulverized to a fine powder with an all-glass mortar and pestle. A portion was weighed and then homogenized at 1000 rpm for 2 min in a Potter-Elvehehm homogenizer with biuret-reagent before measuring protein by the heated biuret-Folin method (Dorsey et al., 1977).

Particulate protein nitrogen. Particulate protein in the algal pools, the clam tank deposits, and the clam-tank effluents were also measured by the heated biuret-Folin method with some modifications. The particulate algal protein samples were filtered onto Gelman 25-mm diameter, 0.45 micron pore-size, glass fiber filters. The entire filter was then heated in biuret reagent for 100 min at 100°C. After this, Folin reagent was

added immediately. After the final color development was complete, the heavy-walled test tubes were centrifuged at 100 x g for 5 min to remove the glass fiber filter before comparing the color with bovine serum albumin standards which were also heated for the same length of time (Dorsey et al., 1977). The absorbance was read at 660 nm in a Gilford model 240 spectrophotometer with 1.0 cm cuvettes.

Inorganic "nitrogen" determinations. The ammonia, nitrite and nitrate concentrations were all determined with a Technicon AutoAnalyzer II, running at 30 samples per hr. The colorimetric assays were done as described in the standard Technicon AutoAnalyzer methodology handbook (methods which are based on procedures given by Strickland and Parsons, 1972). A computerized peak integrator was used to determine the concentrations of these nitrogen-containing compounds.

Results

Deep-Water Nitrate Conversion into Algal Protein

At the 1.1 day^{-1} turnover rates used for Chaetoceros curvisetus (STX-167) and S-1 culture tanks, 69% of the deep-water nitrate nitrogen was converted into phytoplankton protein nitrogen, determined on the mixture of the cultures of the two species taken from the mixing-tank outflow. Sixteen percent of the nitrate remained unaltered, leaving 15% of the incoming nitrate unaccounted for. A good portion of this is probably in the nucleic acids of the phytoplankton cells, some may be present as intracellular nitrate and some organic nitrogen may have been released to the medium. The percentages given in Table 1 are derived from the

particulate protein and nitrate concentrations determined daily and averaged over the entire 36-day period of the experiment.

Table 1

Clam Feeding and Growth

"Percent stripping" is defined as the fraction of the PPN removed in the clam tank from the food inflow. This includes uptake by the clams as well as loss of PPN to the tank deposit. The advantage of using PPN as a measurement, is that algal cells of vastly different sizes or shapes can be quantitatively compared.

The "percent stripping" was constant for most experimental clam groups. For the 35 g, 2 ml/sec group, the individual clam growth rate was the greatest, and for this group, culling on day 18 resulted in reduced percent stripping, as shown in Figure 2.

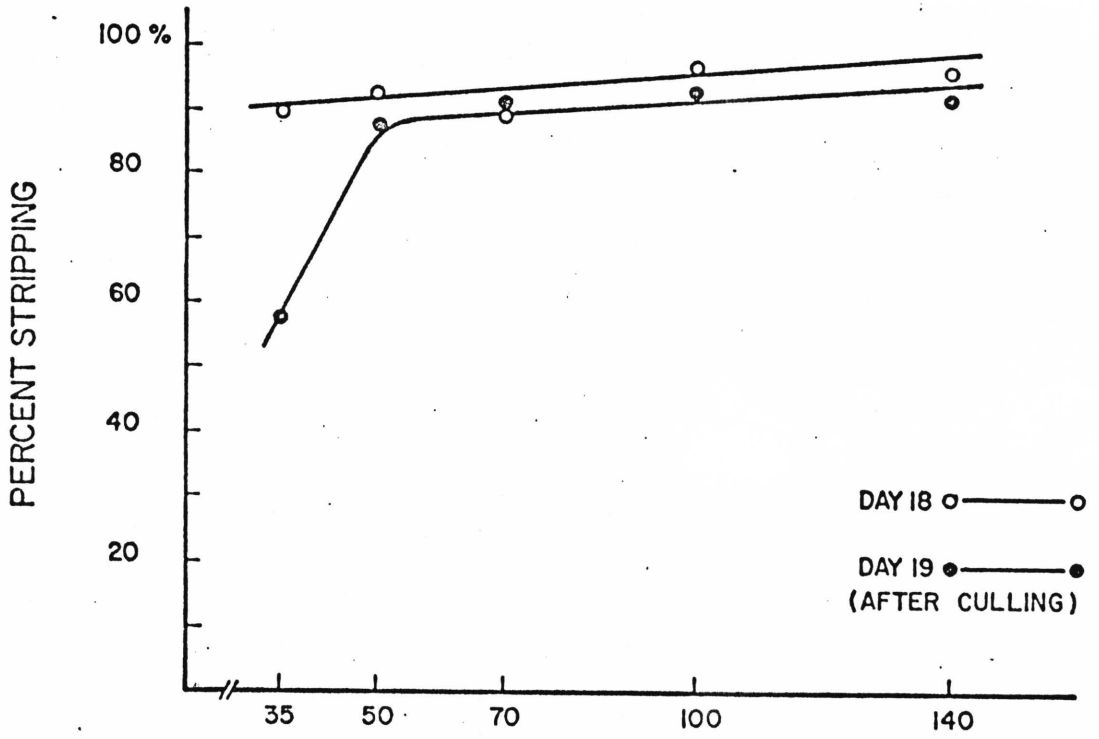
Figure 2

On days 18 and 19 of the experiment, the food concentration flowing into the Tapes feeding tanks was 24.1 and 21.9 µg-at PPN/l, respectively. On day 18, the clam weights were reduced to the original starting weight by culling. Thus, for the 35 g, 2 ml/sec group, culling on day 18 removed half the weight of clams in the tank. Figure 2 shows that the percent stripping was almost the same for all groups, except for the 35 g, 2 ml/sec group on day 19. The percent stripping for this group was lower on the average during the entire experiment: 74% compared to 87-93% stripping for all other groups.

Table 1. The conversion of deep-water nitrate in the phytoplankton culture system by *Chaetoceros curvisetus* (STX-167) and S-1.

Deep-Water Nitrate-Nitrogen Conversion	
Algal protein-nitrogen	69%
Unconverted nitrate-nitrogen	16%
Non-protein nitrogen in algal cells and dissolved organic nitrogen in medium (by difference)	15%

CHANGE IN PERCENT STRIPPING AFTER Tapes CULLING



The increase in length of the different groups of clams over the 36-day experimental period is given in Figure 3.

Figure 3

The 35 g, 2 ml/sec group had the lowest percent stripping and the greatest increase in length, as shown in Figure 3. This figure gives the average increase above the starting length of the clams remaining in the experiment on day 36 of the experiment. The 35 g, 2 ml/sec group grew from 12.7 mm to 19.8 mm, an increase of 7.1 mm, in 36 days. This was the highest average individual growth rate achieved: 1.42 mm/week.

Figure 4 gives the weight gain (g) for each experimental group of Tapes japonica.

Figure 4

The total weight gain is greatest for the 100 g, 2 ml/sec group, which showed an increase in wet weight of 134 g over the 36-day period. Although this population had the greatest weight increase as a group, the growth rate of the individual clams averaged .268 g/week/g whole clam, compared to the 35 g, 2 ml/sec group which hadan average growth rate of .411 g/week/g whole clam.

The composition of the clams varied slightly, but on the average, 47% of the whole wet weight was wet meat, 16% of the wet meat was dry meat, and 42% of the dry meat was protein.

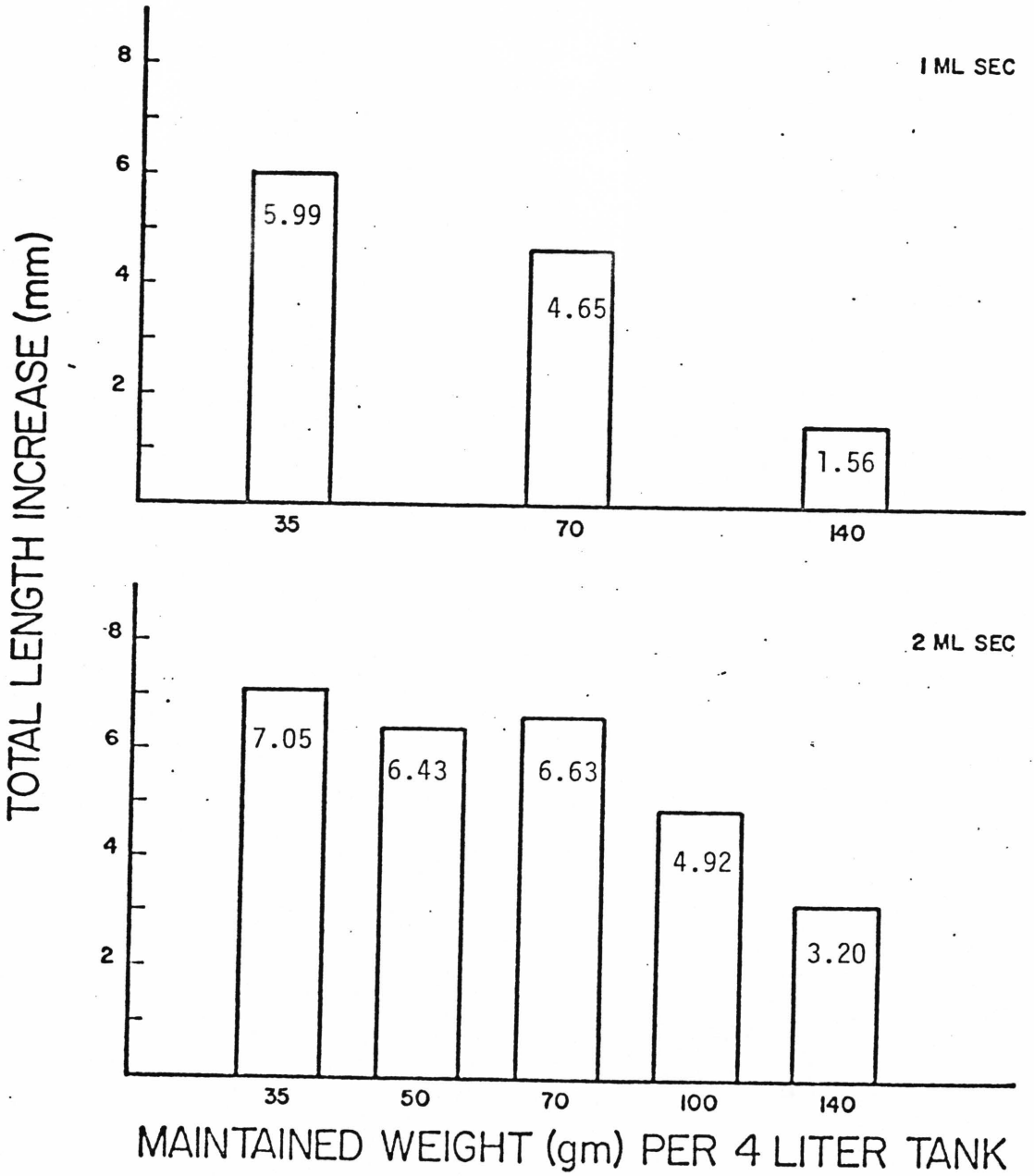
Nitrogen Balance

The nitrogen recovery is shown in Table 2.

Table 2

TOTAL LENGTH INCREASE (mm) OF *Tapes japonica* IN 36 DAYS

STARTING LENGTH 12.7 mm



TOTAL WEIGHT GAIN (gm) IN 36 DAYS

Tapes japonica — FED S-I AND STX-167

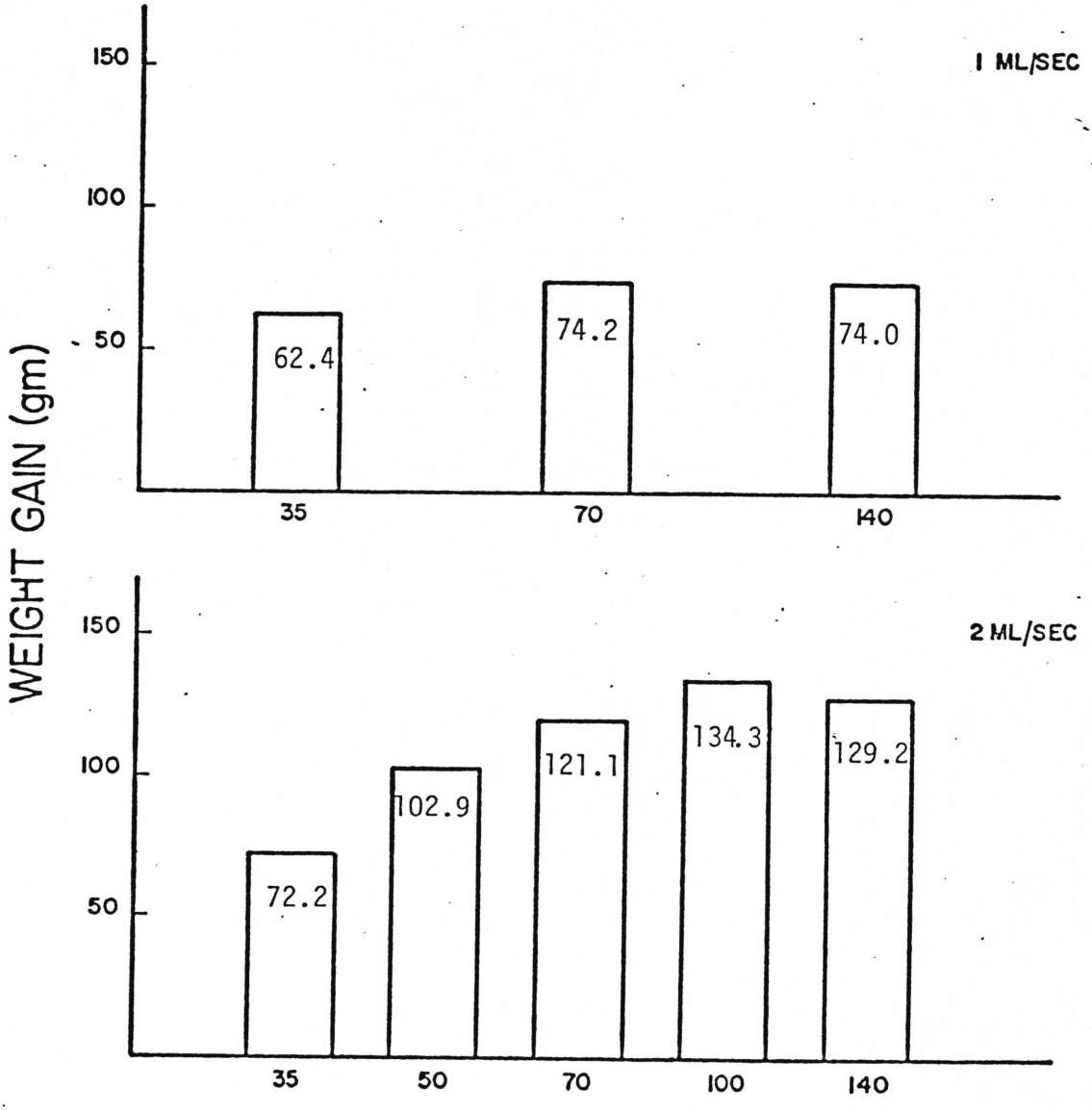


Table 2. The total nitrogen recovered in the shellfish feeding tanks and their effluent as a percentage of the inflowing algal protein nitrogen.

Plankton Suspension Flow Rate	Starting Clam Weight (g)	Total Nitrogen Recovered During 36-Day Period "N" Recovered as % of Algal Protein-N Inflow
1 ml/sec	35	73.9
	70	76.6
	140	88.2
2 ml/sec	35	70.6
	50	72.4
	70	73.1
	100	70.2
	140	71.9

"N" recovered = meat protein-N + shell protein-N + tank deposit protein-N + tank effluent (protein-N + NO_3^--N + NO_2^--N + NH_4^+-N)

The utilization of the algal protein by the clams is shown in Table 3.

Table 3

To trace the fate of algal protein nitrogen (PPN) through the system, the PPN flowing into the shellfish feeding tanks was assigned a value of 100%. The incorporation of this PPN into clam meat protein, tank deposit particulate protein, tank effluent particulate protein, effluent ammonia and effluent (nitrate + nitrite), were then expressed as a percentage of algal PPN in the food flow coming into the shellfish tanks. This percentage value for each fraction was averaged, based on the daily values obtained, over the entire duration of the 36-day experiment. Three to four hours' time was subtracted from each nine-day period while weighing, measuring and culling was taking place.

Figure 5 shows the percentage incorporation of PPN into clam meat protein.

Figure 5

The greatest total amount of protein was incorporated into the shellfish meat by the 100 g, 2 ml/sec group; this group also had the greatest fresh weight gain. Thirty-two percent of inflowing PPN was incorporated into clam meat protein by this group. The rate of protein-nitrogen incorporation was equal to 12.0 µg-at protein N/day per g whole clam for this group. The 35 g, 2 ml/sec group had the least efficient food utilization and the fastest growth of individual clams. This group converted 24% of inflowing algal protein into clam-meat protein, corresponding

Table 3. Protein utilization by *Tapes japonica* of a mixture of *Chaetoceros curvisetus* (STX-167) and S-1.

Weight Density (g/4-l)	Flow Rate (ml/sec)	Total Whole Weight Gain of Clams (g)	Algal Protein in the Inflow (g)	Algal Protein Stripped (g)	Algal Protein Absorbed (g)	Algal Protein Retained (g)	Protein Efficiency Ratio[1]	"Stripped" Protein Efficiency Ratio[2]	Biological Value[3]
35	1	62.4	5.72	5.01	4.58	1.86	10.9	12.5	40.6
70	1	74.2	5.72	5.33	4.97	1.97	13.0	13.9	39.6
140	1	74.0	5.72	5.34	4.96	1.78	12.9	13.9	35.9
35	2	72.2	11.5	8.52	7.75	2.71	6.30	8.47	35.0
50	2	102.0	11.5	10.0	9.24	3.42	8.99	10.2	37.0
70	2	121.0	11.5	10.3	9.56	3.71	10.6	11.6	38.8
100	2	134.0	11.5	10.6	9.84	3.75	11.7	12.7	38.1
140	2	129.0	11.5	10.6	9.95	3.49	11.3	12.1	35.0

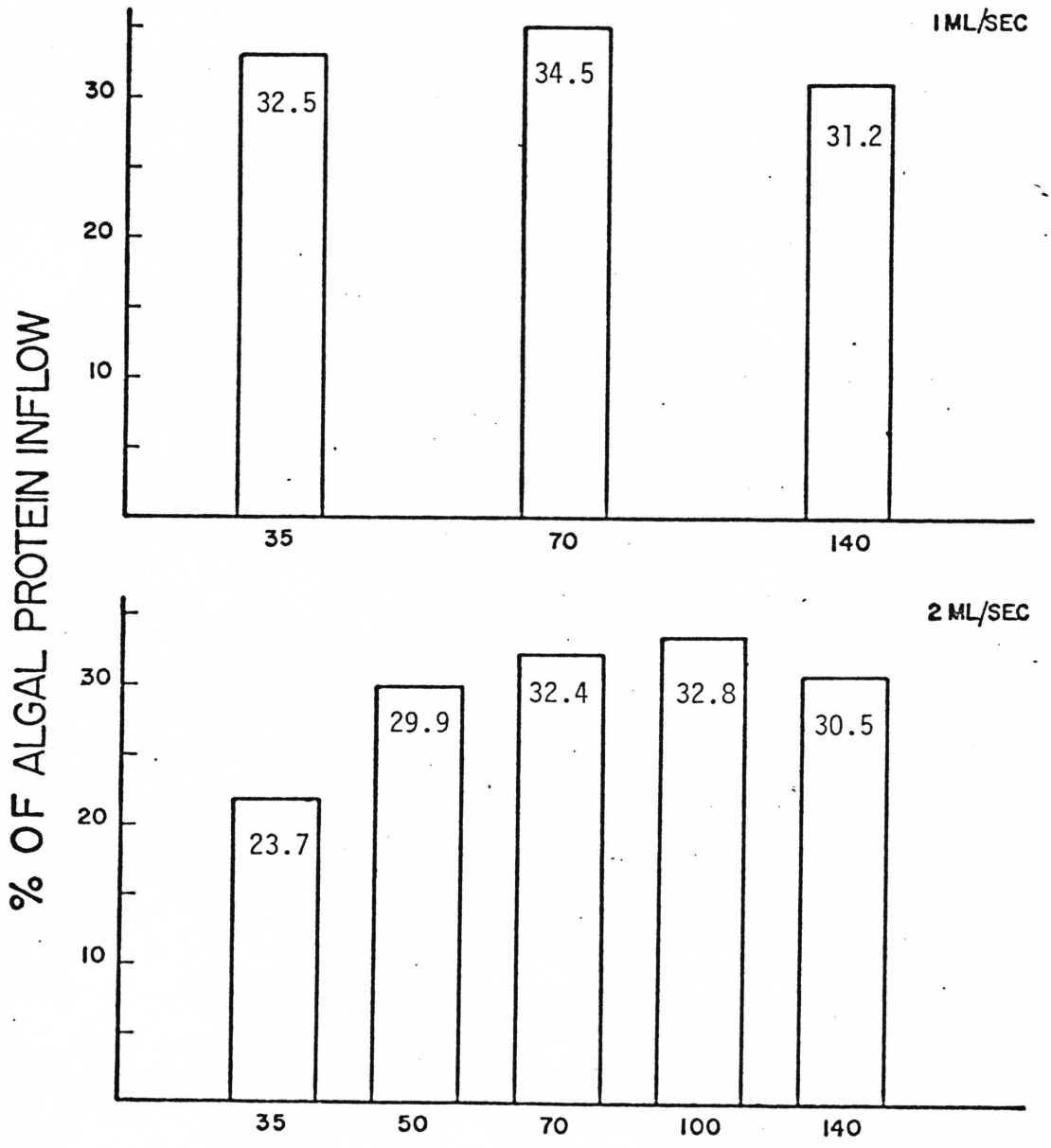
[1]Protein Efficiency Ratio = total weight gain/weight protein presented.

[2]"Stripped" protein efficiency ratio = total weight gain/weight protein stripped.

[3]Biological Value =(retained protein/absorbed protein) x 100.

PROTEIN CONVERSION EFFICIENCY

SHELLFISH MEAT PROTEIN PRODUCED AS % OF ALGAL PROTEIN INFLOW OVER A 36 DAY PERIOD



to an incorporation rate of 24.7 µg-at protein N/day/g whole clam. The 70 g, 1 ml/sec group had the highest efficiency of protein conversion: 34.5%, the highest percent of phytoplankton protein incorporated into shellfish meat protein.

The percentage of inflowing PPN appearing as tank deposit particulate protein and as effluent PPN is shown in Figures 6 and 7.

Figure 6

Figure 7

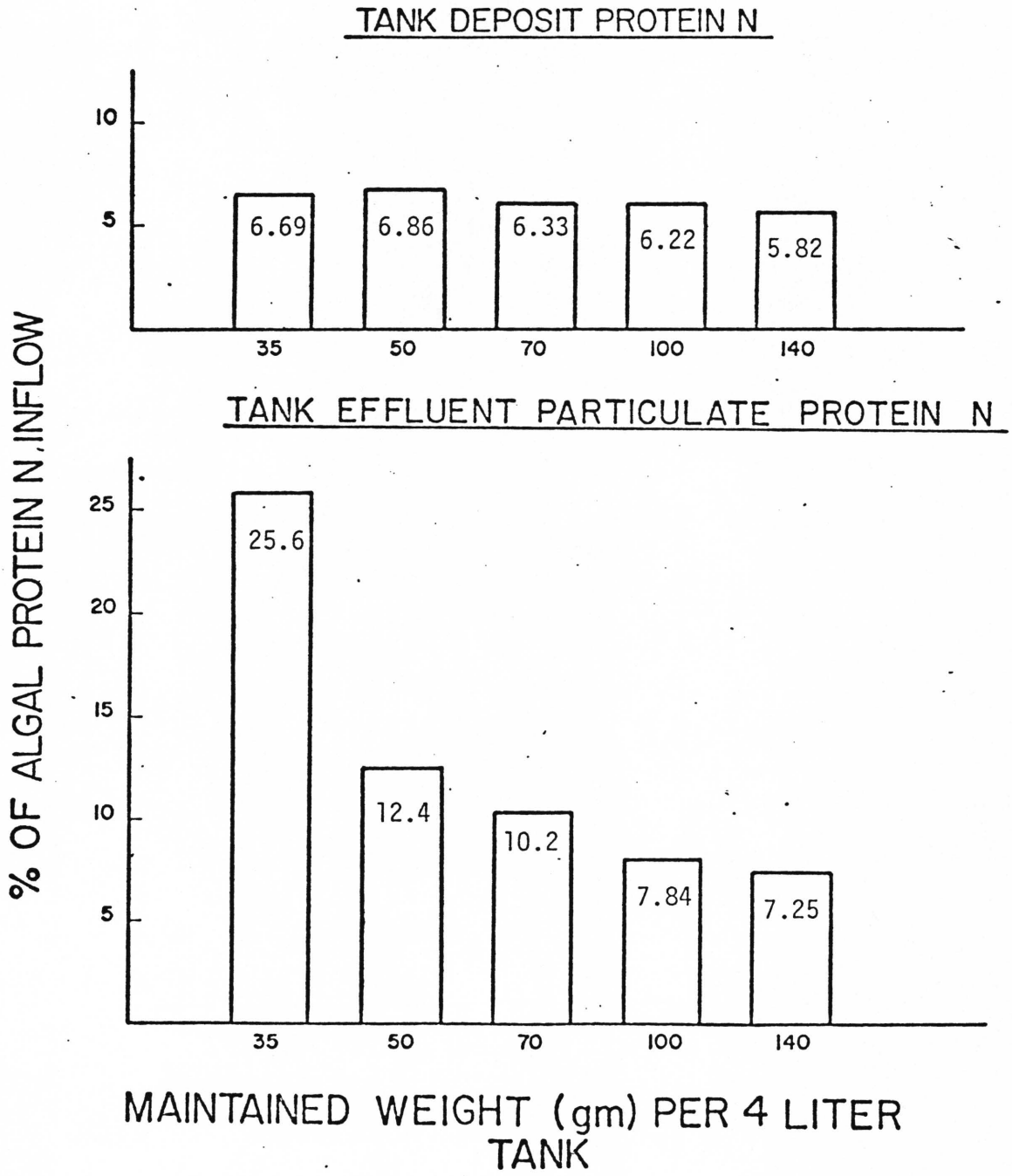
Tank deposit PPN varied little with the clam density or with flow rate and averaged 5-7%.

The amount of particulate protein nitrogen in the effluent varied a great deal among the different experimental groups. Figures 6 and 7 show that a major portion (26%) of the entering protein leaves in the effluent in the least efficient 35 g, 2 ml/sec group. The percentages in Figures 6 and 7 are really equal to 100 minus the percent stripping averaged over the entire period of the experiment. For the entire experimental period, the concentration of PPN in the effluent in µg-at/l was 2.69, 1.48 and 1.45 for the 35, 70 and 140 g, 1 ml/sec groups, and 5.50, 2.67, 2.19, 1.69 and 1.59 for the 35, 50, 70, 100 and 140 g, 2 ml/sec groups, respectively. Only 7.9% of the inflowing PPN remained in the effluent of the group with maximum population growth: 100 g, 2 ml/sec.

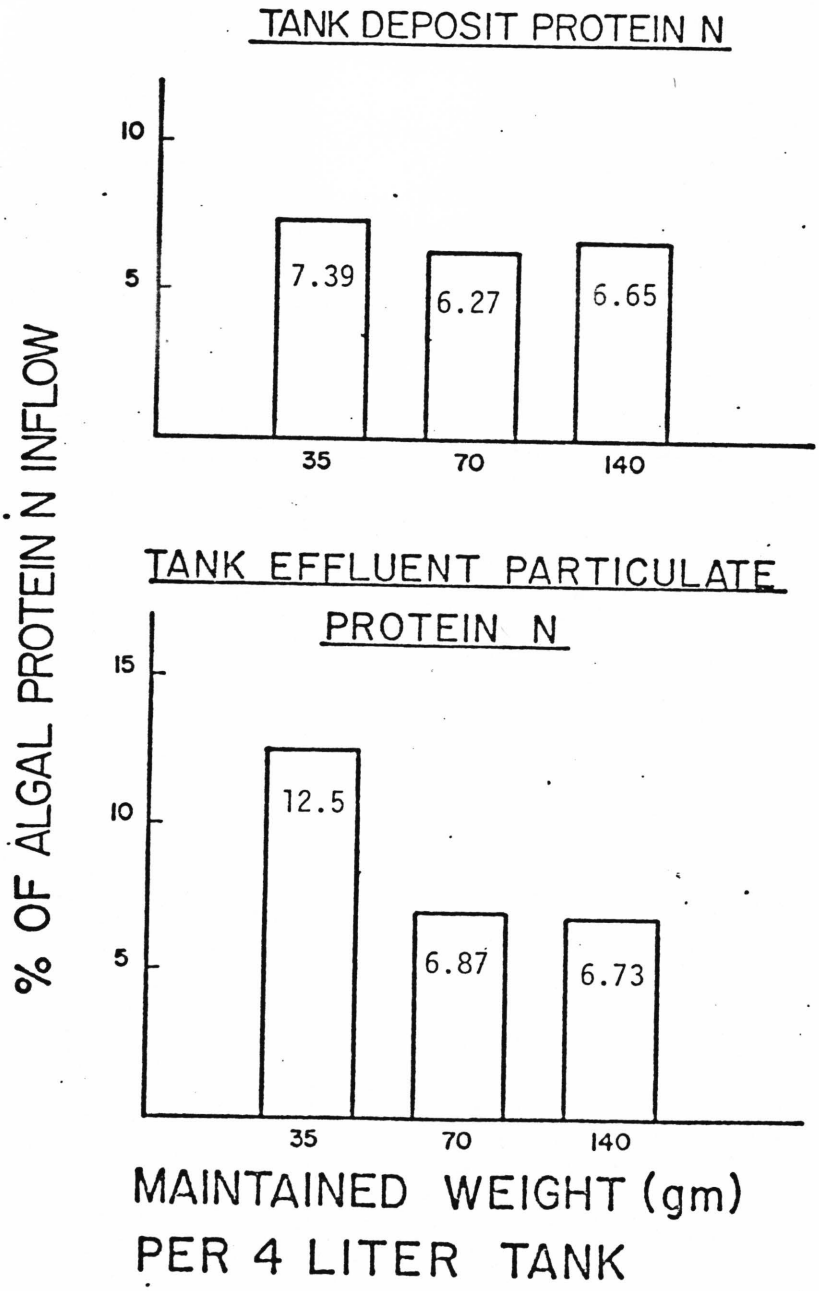
The ammonia-N-concentration in the shellfish tank effluent expressed as a percent of PPN inflow is given in Figure 8.

Figure 8

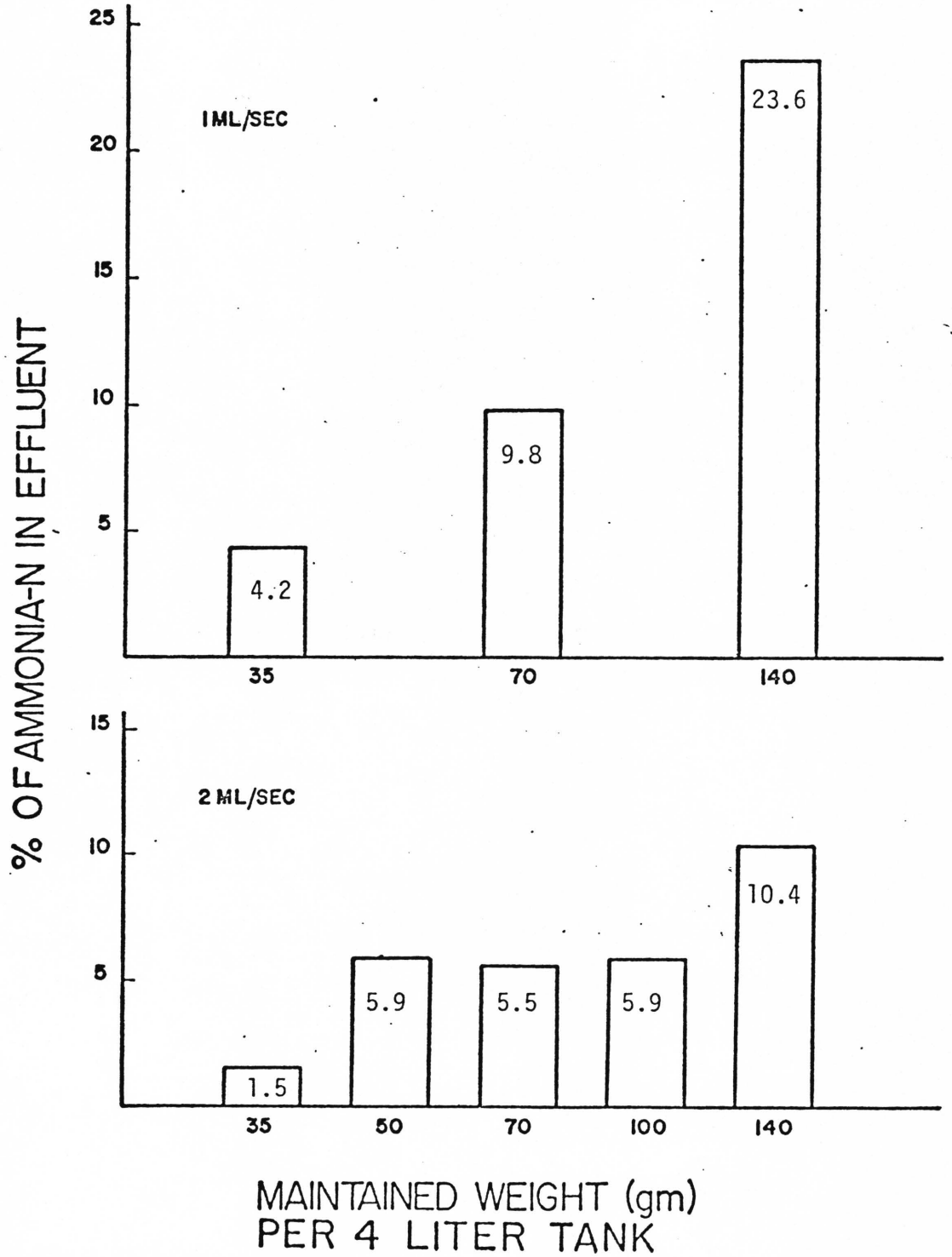
COMPARISON OF TANK DEPOSIT PROTEIN N AND TANK EFFLUENT PROTEIN N AS % OF ALGAL PROTEIN N INFLOW (2 ML/SEC)



COMPARISON OF TANK DEPOSIT PROTEIN N AND TANK EFFLUENT PROTEIN N AS % OF ALGAL PROTEIN N IN INFLOW (IML/SEC)



AMMONIA PRODUCTION AS % OF INFLOWING ALGAL PROTEIN NITROGEN



The absolute NH_4^+ concentrations are 0.91, 2.12 and 5.07 µg-at NH_4^+-N/l for the 35, 70 and 100 g, 1 ml/sec groups, respectively and 0.33, 1.21, 1.20, 1.26 and 2.20 µg-at NH_4^+-N/l for the 35, 50, 70, 100 and 140 g, 2 ml/sec groups generated above the 0.89 µg-at NH_4^+-N/l entering the Tapes feeding tanks. The 140 g, 1 ml/sec group had 23% of its inflowing PPN in the form of ammonia nitrogen in the shellfish-tank effluent, and, although they were actively growing, the growth of the inidividual shellfish was far from maximal for this species.

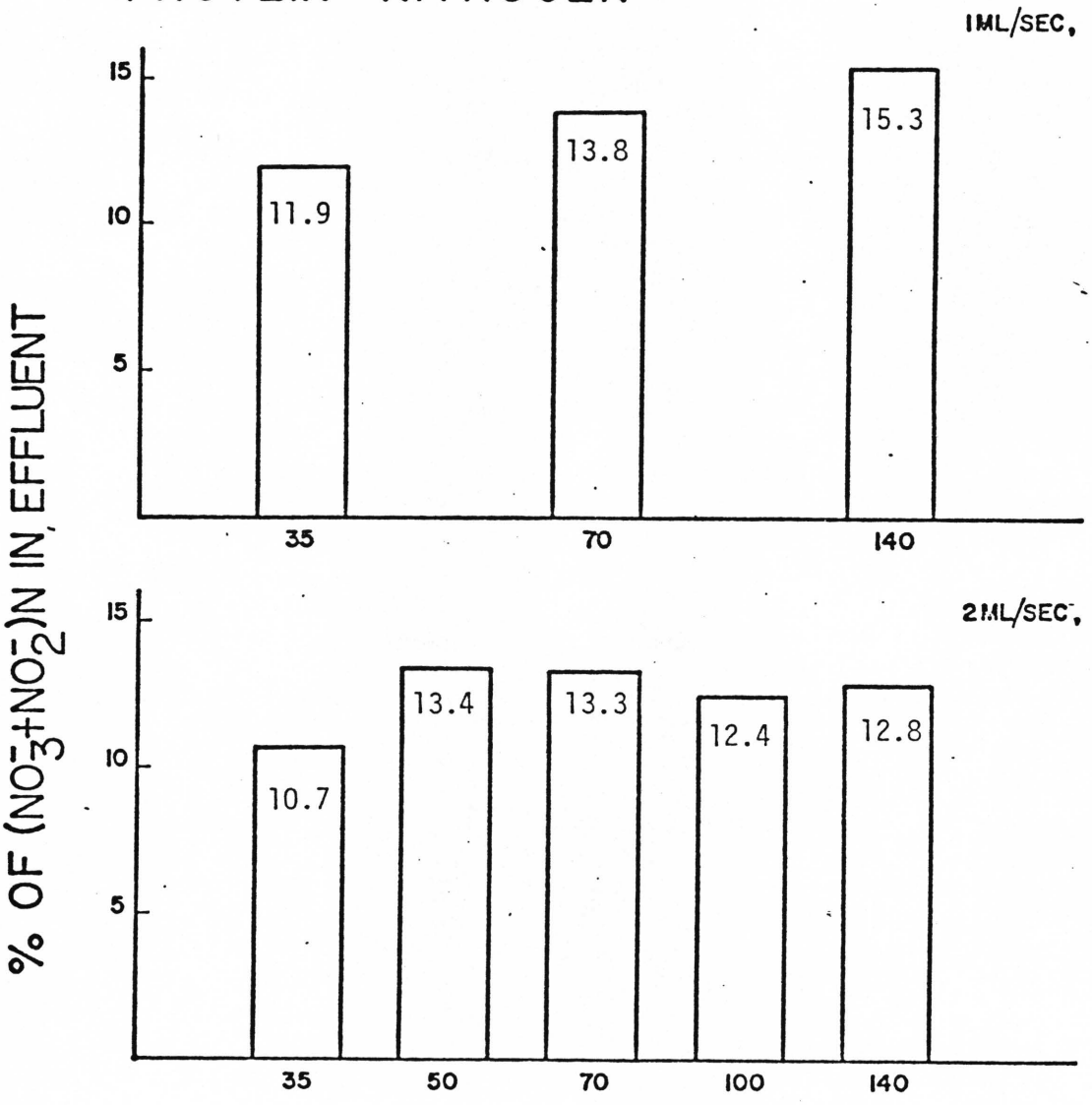
One unexpected result was the amount of NO_3^- produced in the shellfish tanks. As indicated in Figure 9, an amount of NO_3^-

Figure 9

equal to 12-14% of the PPN entering the shellfish tanks was generated in the tanks over and above the original unreacted NO_3^- present in the outflow from the phytoplankton tanks entering the shellfish tanks. The amount of NO_3^- and NO_2^- also did not vary greatly with the weight of the shellfish populations. The most likely explanation for this finding is that nitrifying bacteria are breaking down the tank deposit and converting it into nitrate. Control experiments on tank-deposit protein-nitrogen also show large losses if accumulated tank deposit is allowed to remain in the tanks for several weeks with aeration and no culture flow.

The increase of (NO_2^- + NO_3^-) in the Tapes feeding tanks lies between 2.30 and 3.27 µg-at of combined (NO_2^- + NO_3^-) N/l.

$NO_3^- + NO_2^-$ FORMATION IN THE SHELLFISH TANKS AS % OF INFLOWING ALGAL PROTEIN NITROGEN



The fate of the algal protein nitrogen entering the shellfish tanks for three of the experimental populations is given in Figures 10, 11 and 12.

Figure 10

Figure 11

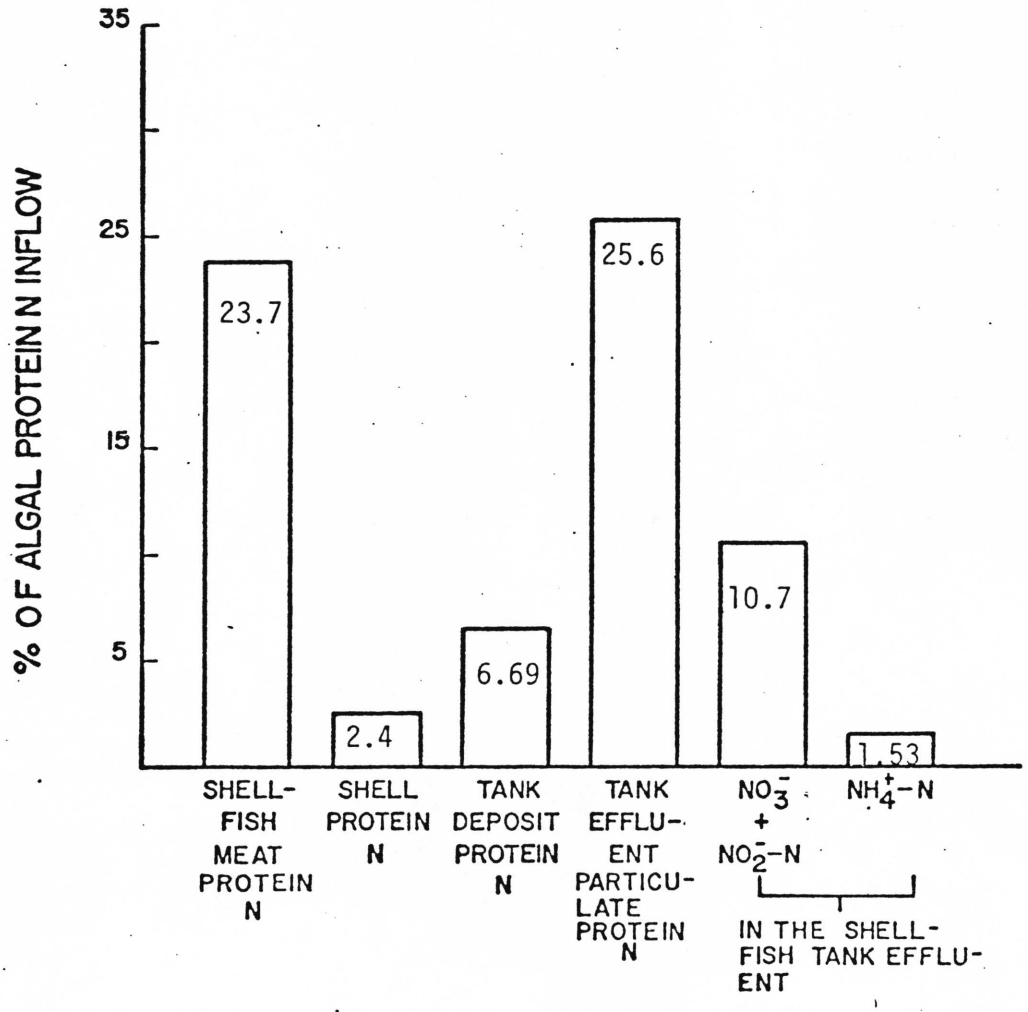
Figure 12

The largest differences appear in the PPN and the NH_4^+-N in the Tapes tank effluent. The highest effluent PPN is found in the group with the fastest individual growth. The highest NH_4+-N is generated by the group with the slowest individual growth.

Discussion

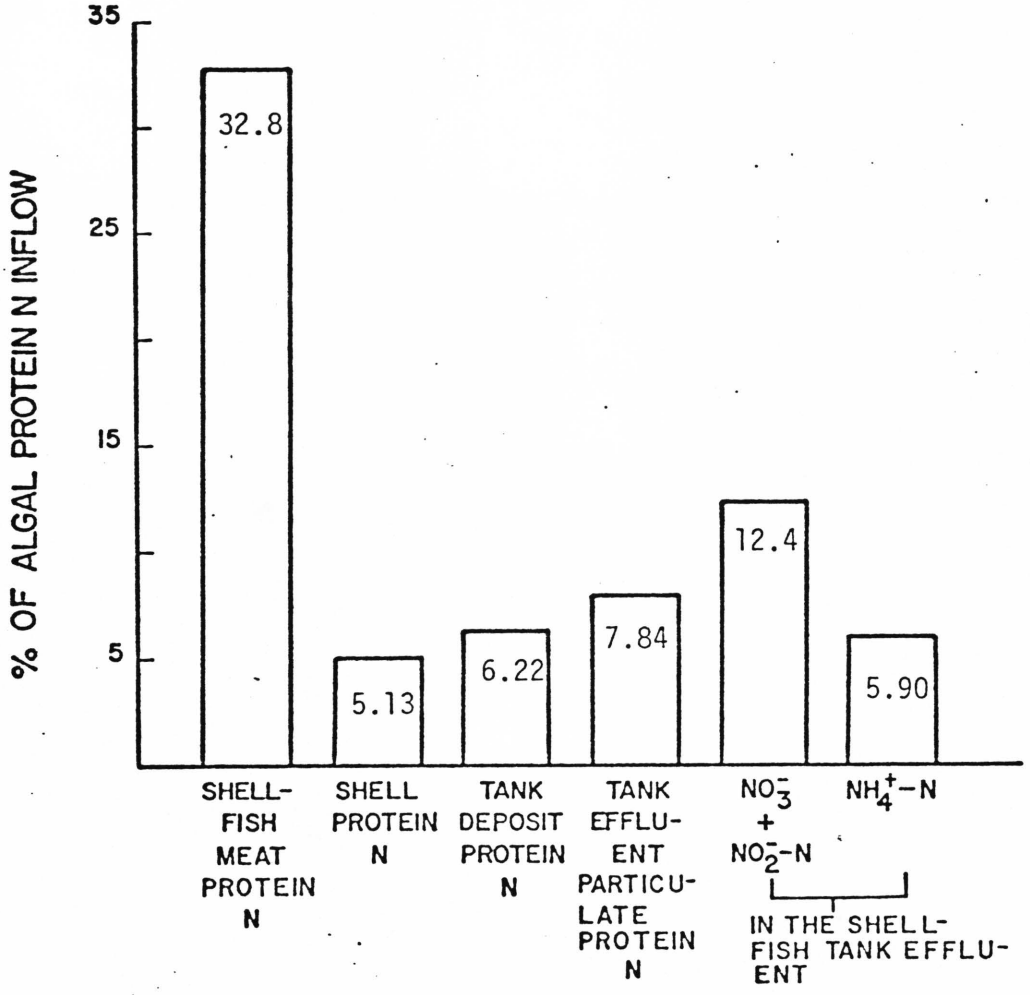
The utilization of deep-water nitrate by the phytoplankton used in this work, Chaetoceros curvisetus (STX-167) and the flagellate S-1, was somewhat lower than normally obtained in the larger-scale mariculture system in St. Croix, where only C. curvisetus (STX-167) is grown. The average conversion of deep-water nitrate-nitrogen into phytoplankton protein-nitrogen by C. curvisetus (STX-167) in the 45,000-liter pools is 78%, corresponding to better than 90% utilization of incoming deep-water nitrate, since the deep-water nitrate is naturally also utilized for the synthesis of nitrogen-containing compounds other than protein. Assuming 70% efficiency of nitrate-nitrogen to phytoplankton protein-nitrogen conversion in our system, the protein production per square meter per year in the St. Croix experimental system, for 330 days' operation of the pools per year, would be 0.52 kg, corresponding to 5.2 tons protein per hectare per year (Roels et al., 1975). By comparison, the best

THE FATE OF ALGAL PROTEIN N FLOWING INTO SHELLFISH TANKS CONTAINING *Tapes japonica*



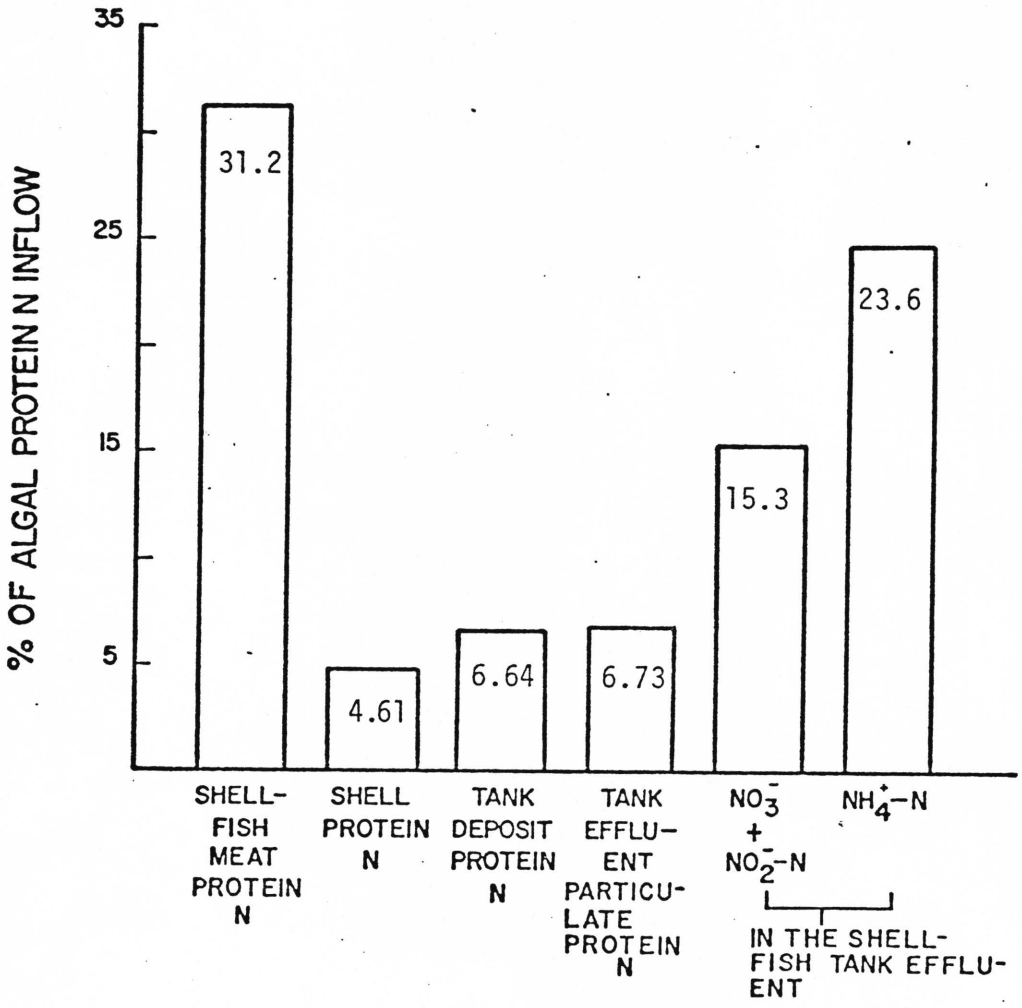
35 GM, 2 ML/SEC

THE FATE OF ALGAL PROTEIN N FLOWING INTO SHELLFISH TANKS CONTAINING Tapes japonica



100 GM, 2 ML/SEC

THE FATE OF ALGAL PROTEIN N FLOWING INTO SHELLFISH TANKS CONTAINING *Tapes japonica*



140 GM, 1ML/SEC

protein output in land-based agriculture is that of alfalfa crops, yielding 0.71 tons protein/ha/yr (Pimentel et al., 1975). The 5.2 tons phytoplankton-protein/ha/yr quoted here applies for a 0.8-m deep pool, as presently used in St. Croix. Extrapolation of experimental results indicates that far higher phytoplankton-protein productivities per unit area could be achieved by using deeper pools (Roels et al., 1976).

The "percent stripping" of incoming phytoplankton in the shellfish tanks was uniformly high for the groups, varying between 87-94%, except for a brief period immediately following each culling, when the percent stripping for the 35 g, 2 ml/sec group was lower (60% on day 19 of the experiment). The 35 g, 2 ml/sec group averaged 74% stripping for the entire experimental period. This group was obviously wasting food. There are several reports in the literature describing the filtration rate of shellfish. These generally show a maximum or optimal filtration rate for different species. Ali (1970), using Hiatella arctica, a suspension filter-feeder, and Winter (1973), using Modiolus modiolus, show a decreasing filtration rate with increasing cell densities. Work with Mytilus edulis by Thompson and Bayne (1972, 1974) and by Tenore and Dunstan (1973), as well as by Forster-Smith (1975) and Schulte (1975), shows that the filtration rate of the mussel reaches a maximum. Forster-Smith (1975) shows the same for Cerastoderma edule and Venerupis pullastra; Tenore and Dunstan (1973) demonstrate it for Crassostrea virginica and Mercenaria mercenaria. This optimum filtration rate must have been reached by the 35 g, 2 ml/sec group for which a marked decrease in stripping was observed. This decrease in percent stripping

might be due to the saturation of the feeding sites on the gills of the clams. There is no indication of ill effects and the 35 g, 2 ml/sec group is growing somewhat faster than the 35 g, 1 ml/sec group. Loosanoff and Engle (1974) have shown that Crassostrea virginica can adapt to cell concentration by pumping at different rates but this condition should have an upper limit.

The 35 g, 2 ml/sec group had the lowest "percent stripping" and the maximal individual growth rate, as shown by the average increase in shell length in Figure 3 and the weight increase per gram of clam. We should stress here that in our experimental design, there is a marked difference in the individual growth of each animal and the mass population growth: Figure 4 clearly demonstrates that the 100 g, 2 ml/sec group of clams showed the greatest increase in total weight over the experimental period. However, the clams in the 35 g, 2 ml/sec group had the greatest individual weight gain and thus would reach market size more quickly. We have therefore determined that optimum shellfish densities differ depending upon whether the greatest growth is desired for the mass population of clams or for the individual clams, or whether maximum food utilization is the goal.

The reason for these differences in growth rate and food utilization is probably the availability of feeding acceptor sites: the clam feeding acceptor sites for the 35 g, 1 ml/sec group are nearly saturated as shown by the fact that doubling the amount of food given to the clams resulted in only moderate increase in growth (Fig. 4). The 35 g, 2 ml/sec group must certainly have all its food-acceptor sites saturated (indicated by reduced percent stripping). This

group had the maximum individual growth. By increasing the clam density in the tanks, i.e., by going from 35 g, 2 ml/sec group to the 140 g, 2 ml/sec group, we are increasing the number of food-acceptor sites while keeping the food constant. The food-acceptor sites at a higher clam density (such as the 100 g, 2 ml/sec group) are not all filled, but a greater number of the sites are accepting food so that the total mass growth is greater but the rate of individual growth is slower.

The results on maximal individual and mass population growth show that if clams are grown in a confined space, small change in clam density affects overall individual and mass population growth.

High clam densities will require high feeding rates for maximum individual growth. If the food concentration (PPN/l) cannot be increased by fertilization because of too high an ammonium ion buildup in the shellfish feeding tanks, higher culture flow rates will be required to maintain maximal individual growth rate. Because water current surrounding the clams affects feeding (Walne, 1972) the current that a clam species can tolerate might well be one of the limiting factors for very high clam densities in an industrial-type mariculture system.

Another purpose of this feeding experiment was to obtain a nitrogen balance on the particulate protein nitrogen entering the shellfish tanks. A complete nitrogen balance for clam feeding is very difficult, since the food and the feces are not separable. However, the fate of the entering PPN was determined, and, on the average, 75% of the entering PPN could be accounted for. At the

clam densities used in this experiment, the efficiency of conversion of phytoplankton protein to shellfish meat protein was equal to 33%, 35% and 31% for the 35, 70, and 140 g, 1 ml/sec flow groups and 24%, 30%, 32%, 33% and 30% for the 35, 50, 70, 100 and 140 g, 2 ml/sec flow groups. The nitrate buildup in the shellfish feeding tanks was constant (11-15%) as was the tank deposit protein (5-7%) for all experimental groups. Only the NH_4^+-N and the PPN in the effluent varied considerably. In fact these variations might become valuable indicators of the growth rate of the clams in a mariculture system. The slowest growing group studied, the 140 g, 1 ml/sec group had high NH_4^+-N concentration in the shellfish tank effluent, equal to 24% of the entering PPN whereas the PPN leaving the shellfish tank was the lowest, i.e., 6.7% of that entering. Conversely, the group with the fastest individual growth had the lowest NH_4^+-N concentration buildup in the effluent, 1.5% of the entering PPN concentration, and the highest PPN remaining in shellfish tank effluent, 26%.

Table 3 shows the protein value of this mixed algal diet for Tapes japonica. This type of information will be important to future nutritional studies. Two classical indicators of protein food value are employed, the Protein Efficiency Ratio and the Biological Value of the diet. Protein absorbed or consumed is the total PPN inflow minus the total PPN in the tank deposit and tank effluent for the duration of the experiment, which is the same as the PPN stripped minus the PPN in the tank deposit. The retained protein-N is the protein nitrogen incorporated into the Tapes meat protein. The definition of Protein Efficiency Ratio and Biological Value is given by the following equations:

$$\text{Protein Efficiency Ratio (P.E.R.)} = \frac{\text{weight gain (g)}}{\text{protein consumed (g)}}$$ (Osborn et al. 1919)

$$\text{The Biological Value (B.V.)} = \frac{\text{Retained protein N} \times 100}{\text{Absorbed protein N}}$$ (Mitchell 1924)

Problems inherent in making comparisons of the biological value of protein in food such as the level of protein and caloric intake are given by Forbes et al. (1956), Rosenthal and Allison (1951), Hegsted and Worcester (1947), and Sherwood and Weldon (1953).

The Protein Efficiency Ratios obtained in our feeding studies (6.30 - 13.0) are higher than those obtained in studies with land animals for which values between 2.0 and 4.0 were found by Morrison and Campbell, by Derse, and by Rosenberg as reported by Campbell (1961). Higher P.E.R. values might be expected in clam feeding studies because of the weight of the shells which are low in protein and also because of the higher water content of clam meat compared to the meat of most land animals.

Our P.E.R. values however do compare well with those obtained on mice fed egg protein, peanut flour protein and wheat gluten as is described by Barnes and Bosshardt, who obtained values between 5 and 12, with a diet containing approximately 2% nitrogen.

An advantage of determining Protein Efficiency Ratios over Biological Value figures is that the P.E.R.s do not require killing the animals to determine the amount of retained protein as do the B.V. data. The Protein Efficiency ratio does require determination of the tank deposit protein nitrogen for the entire experimental period. Since this determination does have some unpleasant drawbacks, a "Stripped" Protein Efficiency Ratio as given in Table 3

might well have as much meaning without necessitating the tedious measurements of protein nitrogen on the tank deposit. We certainly recommend the use of the stripped Protein Efficiency Ratio for more routine work.

The information on the nutritional value of the phytoplankton obtained in this experiment with Tapes japonica is a consequence of using protein-N to quantify the efficiency of food transfer in this marine food chain and represents significant progress over dry weight used in the past to describe the efficiency of phytoplankton conversion to shellfish.

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FIGURE CAPTIONS

Figure 1. A schematic diagram of the constant weight clam-feeding experiment.

Figure 2. The effect of culling on the "percent stripping" in the constant weight experiment.

Figure 3. Average clam length increase (mm) over the 36-day experimental period.

Figure 4. Total weight increase (g) over the 36-day experimental period.

Figure 5. The percent incorporation of algal protein into Tapes meat protein.

Figure 6. Comparison of the percent incorporation of algal protein into tank-deposit and tank-effluent protein for the 2 ml/sec flow groups.

Figure 7. Comparison of the percent incorporation of algal protein into tank-deposit and tank-effluent protein for the 1 ml/sec flow groups.

APPENDIX C

HATCHERY OPERATING MANUAL

FOR THE "ARTIFICIAL UPWELLING" MARICULTURE PROJECT

AT THE

ST. CROIX MARINE STATION

THE UNIVERSITY OF TEXAS MARINE SCIENCE INSTITUTE

JUDITH B. SUNDERLIN

PHYLLIS T. BAAB

C O N T E N T S

I. BROOD STOCK

Brood stock shellfish, usually those introduced as juveniles and grown to market size in the system, are maintained in the "Artificial Upwelling" mariculture system at ambient temperature. These shellfish are continuously fed algal cultures from the 2000-liter reactors. Turnover periods in the rearing tanks range from 90 to 120 minutes. The yearly temperature range in the brood stock tanks is 22°C to 27°C.

New Tapes japonica brood stock, from either California or Washington State, are introduced into the system every six to nine months. This introduction should improve the genetic strain and help avoid inbreeding in this species.

Several days before spawning is attempted, two to three brood stock animals are sacrificed and the gonads examined for ripeness. Selected numbers of the following populations: Tapes japonica, Crassostrea gigas, and the C. gigas Kumamoto variety—are usually ripe throughout the year in the "Artificial Upwelling" system.

II. SPAWNING

Equipment

The following is a list of the equipment used in spawning procedures at the St. Croix "Artificial Upwelling" mariculture project.

a) Shellfish shucking knife

b) Glass-lined, gas-fired water heater

c) Spawning table, 2 ft x 4 ft (0.6 m x 1.2 m) —minimum size

d) Vexar screening, 3/4" (19 mm) mesh, two layers on bottom of spawning table to allow for water circulation under the spawning dishes

e) Pyrex loaf pans, 1.4-liter capacity. For a 2 ft x 4 ft (0.6 m x 1.2 m) table, 14 pans are required

f) Thermometers (4)

g) Beakers, 50 ml (3)

h) Pasteur pipettes and bulbs

i) Sieves, 25.4 cm in diameter, made from Nitex screening ranging in mesh size from 35 to 350 μ, stretched and glued to acrylic tubing

j) 1,2 Dichloroethane (when mixed with acrylic dust, is an excellent glue for the sieves)

k) Polyethylene buckets: 12-, 17-, and 21-liter capacity (a total of 10)

l) Disposable pipettes, 1 ml, for larval counts

m) Alcohol: 10 drops to each 1 ml larval sample

n) Sedgwick-Rafter cells (2 or more)

o) Microscope (100X magnification)

Spawning Procedure

Shellfish brood stock animals are subjected to thermal and chemical stimulation to induce spawning. The following technique has been used to induce spawning in four species of shellfish (Tapes japonica, Crassostrea gigas, C. gigas Kumamoto, and Pinctada martensii).

Adult shellfish are placed in Pyrex spawning dishes (1.4 liter) filled with algal culture, and deep water (22°C to 24°C) is circulated around the spawning dishes one-half hour prior to thermal and chemical stimulation (Fig. 1). To induce spawning, hot deep water is circulated through the spawning table and within 10 mins 31-32°C is reached in the Pyrex dishes. Immediately after reaching 31-32°C cold deep water is circulated through the spawning table and in approximately 45 mins the spawning dishes reach 22-24°C. At the high temperature range, a stripped gonad solution (sperm or eggs) is added to each dish and after 45 mins, if no spawning is observed, this thermal cycle is repeated. Deep water is heated in a glass-lined, gas-fired water heater (Fig. 2).

Figure 1. Spawning table (2 ft x 4 ft; 0.6 m x 1.2 m) containing 14 Pyrex spawning dishes. Four thermometers are used to check for uniform water temperature in the spawning dishes.

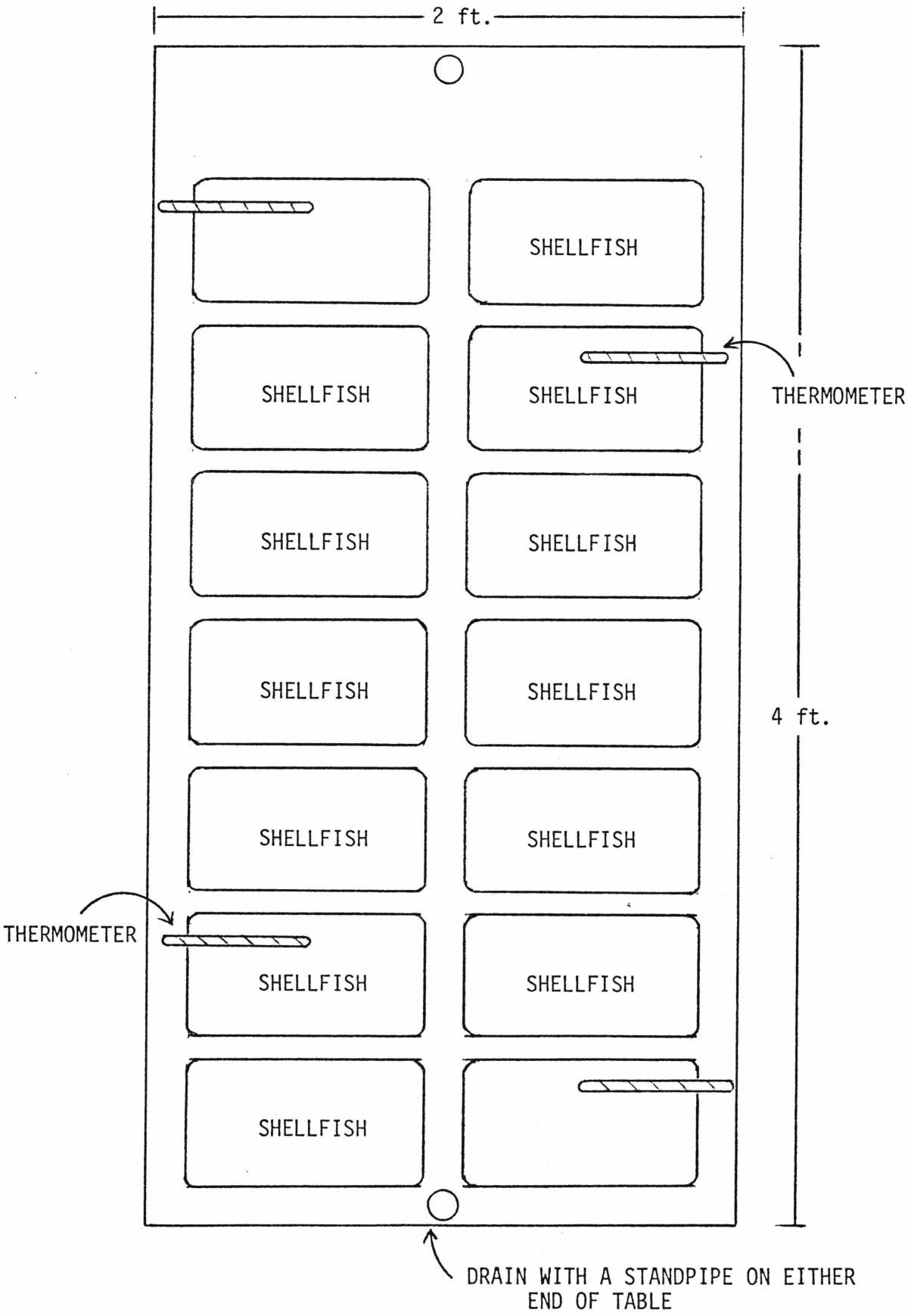
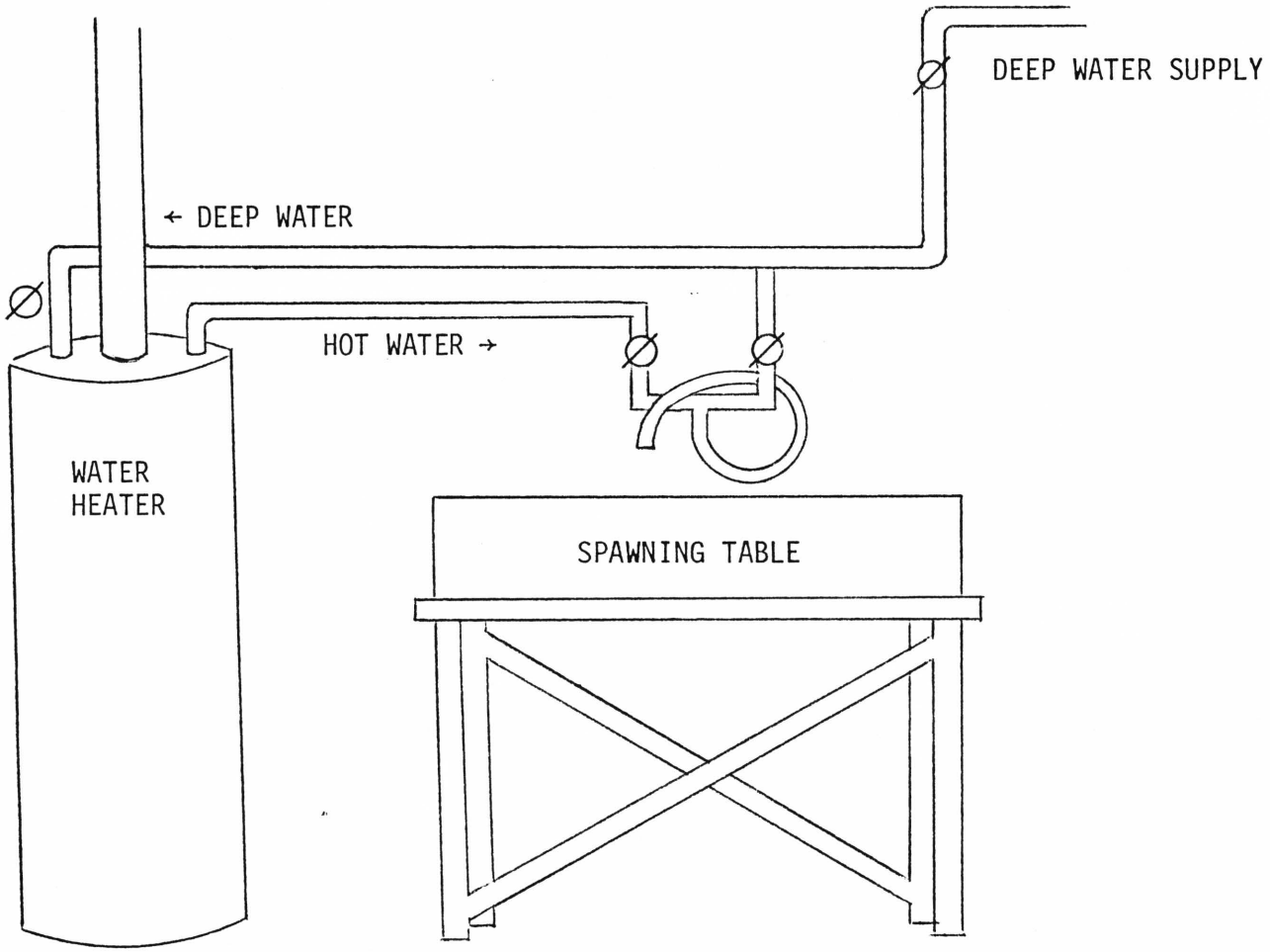

2 ft.
SHELLFISH
SHELLFISH
SHELLFISH
SHELLFISH
SHELLFISH
SHELLFISH
SHELLFISH
SHELLFISH
SHELLFISH
SHELLFISH
SHELLFISH
SHELLFISH
THERMOMETER
THERMOMETER
4 ft.
DRAIN WITH A STANDPIPE ON EITHER END OF TABLE

Figure 2. Thermal stimulation to induce spawning in shellfish. Deep water is heated in a glass-lined, gas-fired water heater to a temperature of 31-32°C.


DEEP WATER SUPPLY
← DEEP WATER
HOT WATER →
WATER
HEATER
SPAWNING TABLE

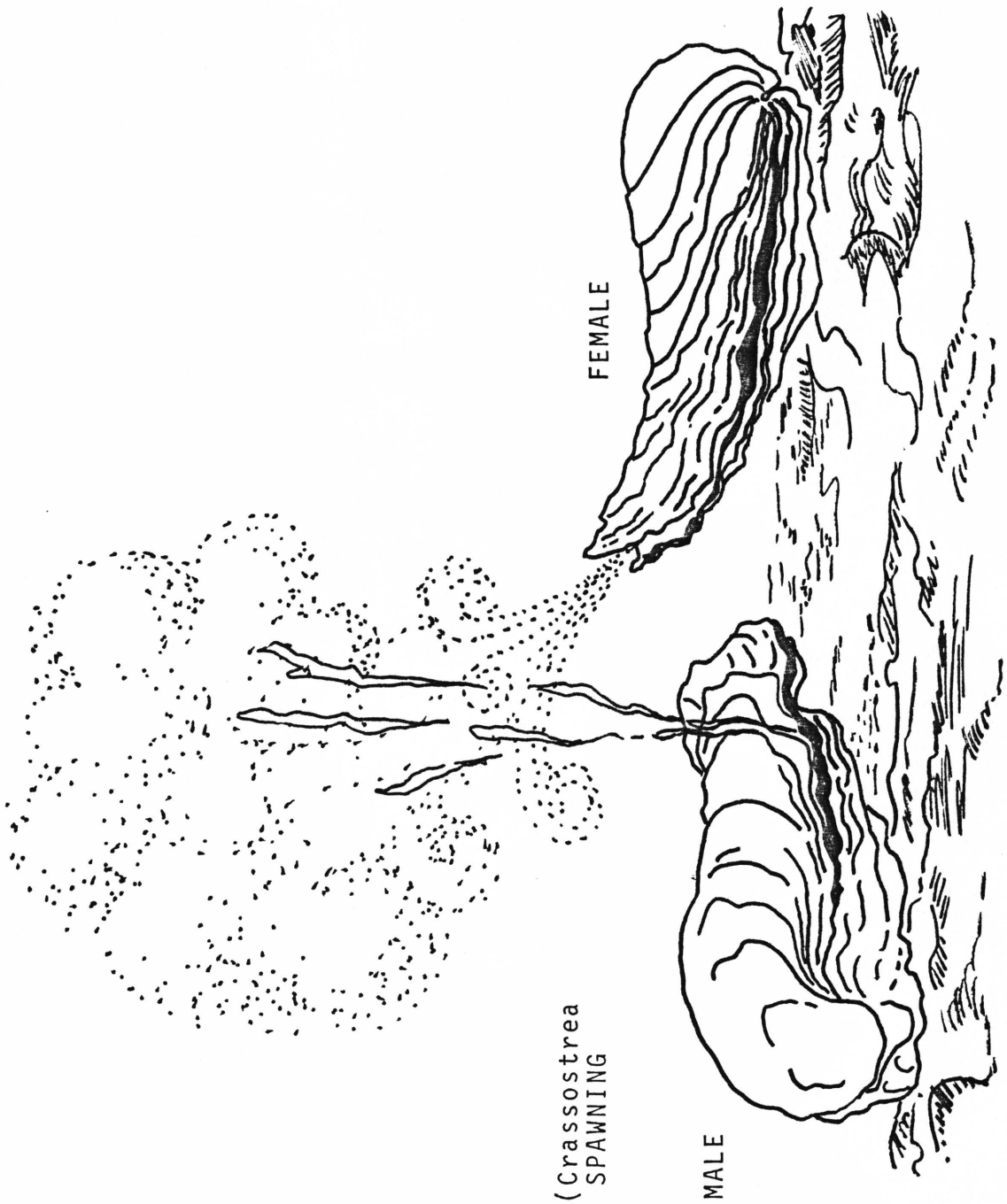
After two or three thermal cycles, if no spawning occurs, all the water is siphoned out of the dishes and they are refilled with algal culture. Then a heat shock is given; the shellfish usually spawn after this treatment.

If spawning is induced, it is important to isolate any spawning male from dishes that may contain females. Therefore, two Pyrex dishes containing algal culture are devoid of shellfish during spawning attempts and are ready to accommodate spawning males. We have found that too much excess sperm in a dish or bucket of fertilized eggs may interfere with cleavage and irregular larvae are produced.

The sex of the clam or oyster can be determined only at time of spawning or only after the shellfish has been sacrificed. Male oysters (C. gigas and Kumamoto) release sperm in a thin, erratic stream from the area of the excurrent siphon. Female oysters release a cloud of eggs by opening and closing the valves almost with a clapping motion (Fig. 3).

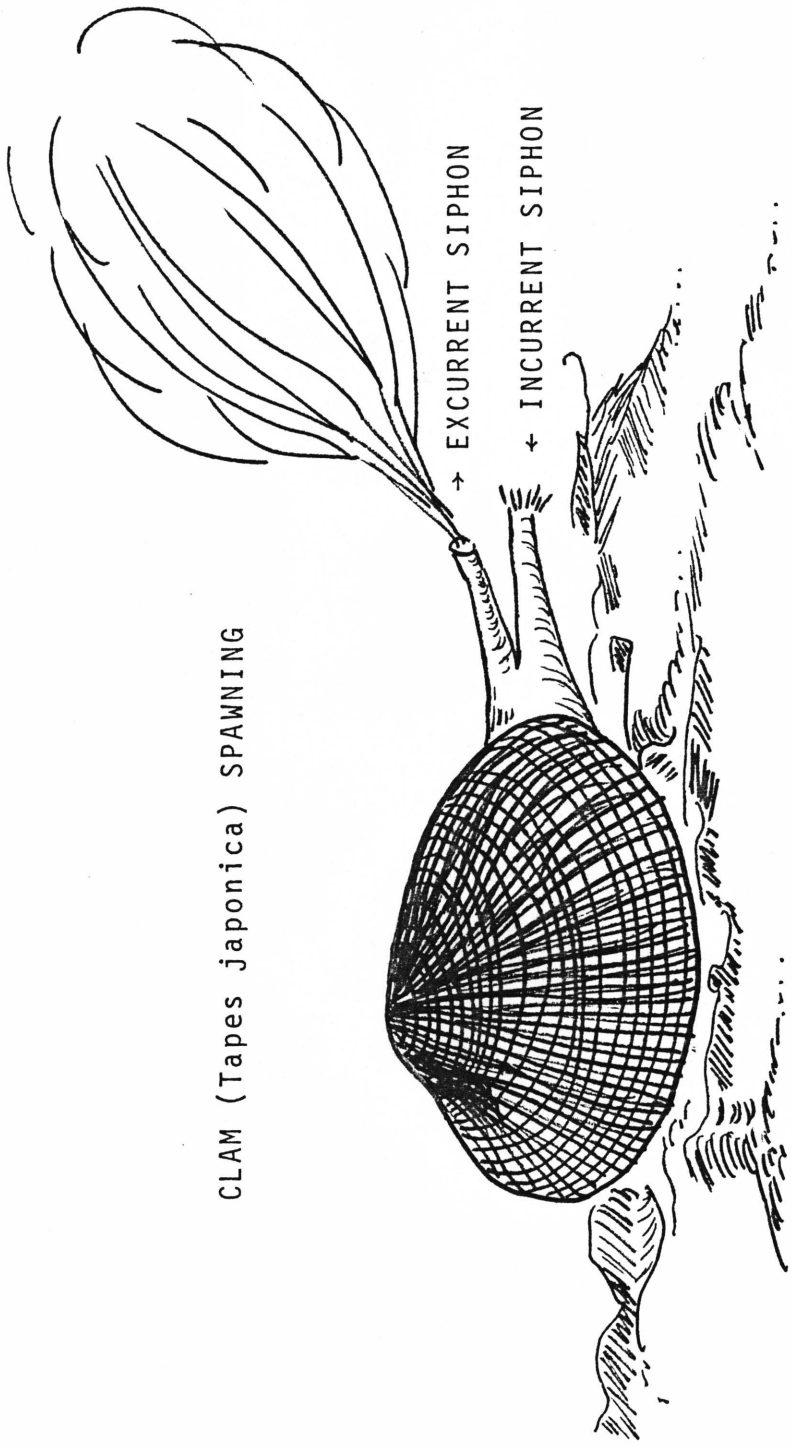
Tapes japonica release sex products through the excurrent siphon (Fig. 4). Sperm released into the water gives a milky appearance to the spawning dish; eggs released in the water are observed as tiny, individual specks that will eventually settle out on the bottom of the dish. The observations of sperm and eggs in the water are similar for both oysters and clams.

Figure 3. Male oysters (Crassostrea gigas and C. gigas Kumamoto variety) release sperm in a thin erratic stream from the area of the excurrent siphon; females release a cloud of eggs by opening and closing the valves almost with a clapping motion.



OYSTERS (Crassostrea gigas) SPAWNING

Figure 4. Tapes japonica clams release sex products through the excurrent siphon. Sperm released into the water gives a milky appearance; eggs appear as tiny, individual specks.


CLAM (Tapes japonica) SPAWNING
→ EXCURRENT SIPHON
← INCURRENT SIPHON

After female shellfish have been induced to spawn, the eggs are siphoned out of the spawning dishes into a bucket containing approximately 3-4 liters of deep water. Please note—when siphoning out the eggs, care should be taken to avoid draining all the water out of the dish and exposing the spawning female. After siphoning, refill the dish with water of comparable temperature. As long as the female continues to spawn, repeat the siphoning and refilling steps to collect as many eggs as possible.

Fertilize the eggs with approximately 10-15 ml of sperm solution (color of watered-down milk). If sperm is less dense, use 5-10 ml more for fertilization. After the sperm solution has been added, allow the bucket of eggs (with volume of 10-12 liters) to stand, with one air-line bubbling slowly, for 30 mins. Then filter the contents of the bucket through a 130μ sieve. This sieve will collect any debris or clumped gonad material and allow the clean, fertilized egg solution to pass through. Tapes eggs are approximately 61μ in diameter and C. gigas and Kumamoto oyster eggs about 48μ.

Note—If Tapes spawn spontaneously in a tank, the eggs can be filtered through a 130μ sieve (to remove debris) and onto a 35μ sieve; the eggs will be caught on the 35μ sieve but the extra sperm will pass through. Several batches of Tapes have been reared after collecting cleaving eggs on a 35μ sieve and growth was normal.

After filtering through a 130μ sieve, the fertilized

eggs are randomized and sampled (a 15 ml sample is taken) for counting and observation. A Sedgwick-Rafter cell is used to determine the number of fertilized eggs (or larvae) per milliliter; duplicate or triplicate 1-ml aliquots of the sample are counted.

The total number of fertilized eggs can be calculated if the volume of the fertilized egg concentrate is known.

Example of a calculation:

16-liter concentrate of fertilized eggs

Counts: 1. 212 eggs/ml

2. 193 eggs/ml

Average # eggs/ml = 203

or $\frac{(212+193)}{2} = 203$

Total # eggs in concentrate = 3,248,000

or (16,000 ml x 203/ml = 3,248,000)

The eggs are then dispersed into either 15-, 30-, 50-, or 379-liter polyethylene or fiberglass containers at 15-20/ml for oysters and 10-15/ml for Tapes clams. These containers are slowly aerated at all times.

Algal culture, previously filtered through a 35μ sieve, is added on Day 0 to the polyethylene containers to achieve a concentration of approximately 5×10^4 cells/ml since straight-hinge, veliger larvae develop within the first 24 hr. The larvae begin to feed at this point in their cycle and the first filtration and regular feeding are not scheduled until 36-48 hr after spawning. The reason for the delay in regular filtering is to ensure complete development

of the larval shell. If larvae are filtered too early, the shell may be injured and deformities and eventually mortality occurs (Loosanoff and Davis, 1963).

Word of caution! After spawning has been attempted using thermal and chemical stimulation, the brood stock animals should NEVER be placed back into the tank containing the other brood stock. This rule should be followed even if attempts at spawning are not successful. There is always a chance that the brood stock you were working with will spawn late in the day. Thus, if they were put back with the others all your brood stock may spawn and put you out of business for a while. Therefore, brood stock used in the hatchery should be placed in a separate pan with a continuous food supply for 4-5 days to ensure that no spawning will occur.

III. LARVAL REARING AND FEEDING

Rearing of Larvae

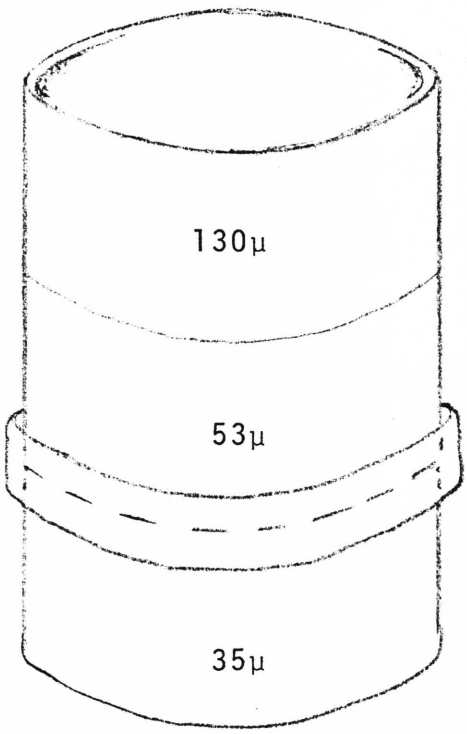
The following will be a basic description for rearing all bivalve larvae in the "Artificial Upwelling" mariculture system. If there is an exception for a particular species, it will be mentioned.

Every other day, the larval cultures are filtered through a graduated series of three 25.4-cm diameter Nitex sieves (made with nylon-monofilament bolting cloth; Tobler Ernst, Fraber Inc., Elmsford, New York)(see Fig. 5). In the top sieve, clumped food and debris are trapped and discarded while larvae are collected on the bottom two sieves. The larvae are rinsed from the sieves and combined in a concentrate of 3 liters or more, depending on the number of larvae present; the concentrate should have at least 30 larvae/ml.

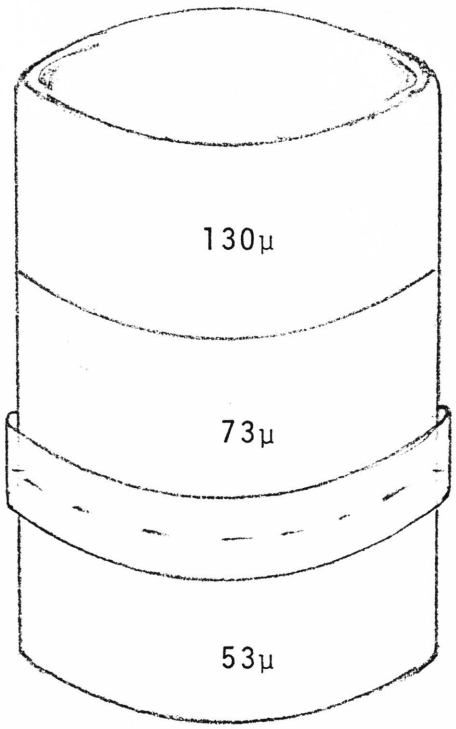
Sieve sizes are increased on the next filtration day if a large percentage (90% or more) of the larvae accumulate on the middle sieve. When selecting sieve sizes, be sure to check the "diagonal" of the specific mesh size being used. In all cases, the diagonal is considerably larger than the mesh size recorded on the sieves and unless care is taken in selecting sieves, many larvae may be lost. Table 1 lists sieve mesh size and the corresponding diagonal in microns.

A 15-ml sample of the larval concentrate is taken after

Figure 5. Series of sieves used to separate different size fractions of oyster larvae and of clam larvae. The 130μ sieve fits loosely on top of the middle sieve to allow air to pass, but the bottom two sieves are aligned with each other using a plastic collar.



FIRST SERIES OF SIEVES
FOR OYSTER LARVAE
(C. gigas & Kumamoto)



FIRST SERIES OF SIEVES
FOR CLAM LARVAE
(Tapes japonica)

TABLE 1. HATCHERY SIEVE SIZES

MESH SIZE IN MICRONS (MARKED ON SIEVES)	CORRESPONDING DIAGONAL IN MICRONS
350	495
300	424
253	358
211	293
163	231
130	184
102	144
86	122
73	103
53	75
35	49
20	28

thorough randomization (Fig. 6). Procedure for randomizing the larval concentrate is to gently plunge the "randomizer" up and down in the bucket at least 10 times, taking care not to touch the "randomizer" to the bottom of the bucket and not to splash the contents of the bucket.

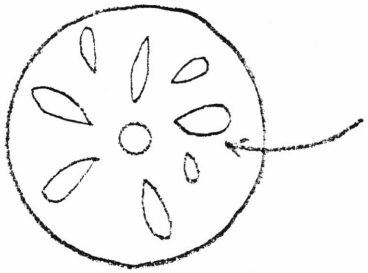
Data on larval growth and survival are obtained from the 15-ml sample. A Sedgwick-Rafter cell is used to determine the number of larvae per ml and either duplicate or triplicate 1-ml aliquots of each sample are counted on Day 2 and Day 10. At least 10 larvae from each sample are measured (each time the larvae are filtered)—length and width in microns (Loosanoff et al., 1966)—using an ocular micrometer (Fig. 7).

Feeding of Larvae

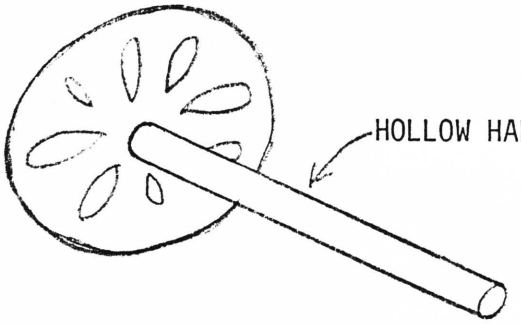
All algal culture should be filtered through a 35μ sieve before introducing it into the larval cultures. The source of the algal cultures (i.e., Pool #__, Reactor #__, Polytank #__, or Carboy dated _____) and the quantity used should be recorded in the notebook each time the larval culture is filtered. The initial number of algal cells/ml added to the larval containers is determined either by cell counts or extrapolation from turbidity measurements. The initial food concentration in the larval cultures ranges from 8×10^4 to 2×10^5 cells/ml. Larvae fed a mixture of algal species reach setting size faster than those fed diets of monocultures. For Tapes, the mixture of 3H, STX-114, and S-1 (two diatoms and a naked flagellate: Thalassiosira

Figure 6. A randomized sample of larvae is taken by gently plunging the "randomizer" up and down in the bucket at least 10 times, taking care not to touch the "randomizer" to the bottom of the bucket and not to splash the contents of the bucket.

THE "RANDOMIZER"



CUTOUTS IN POLYETHYLENE BASE



HOLLOW HANDLE (APPROX. 15" or 38 cm LONG) ATTACHES TO CENTER

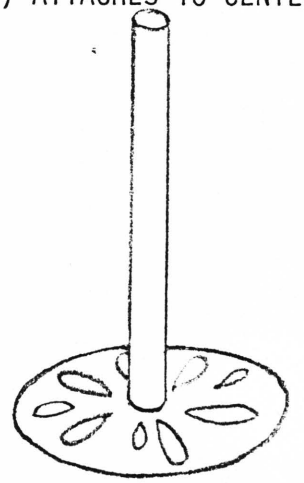


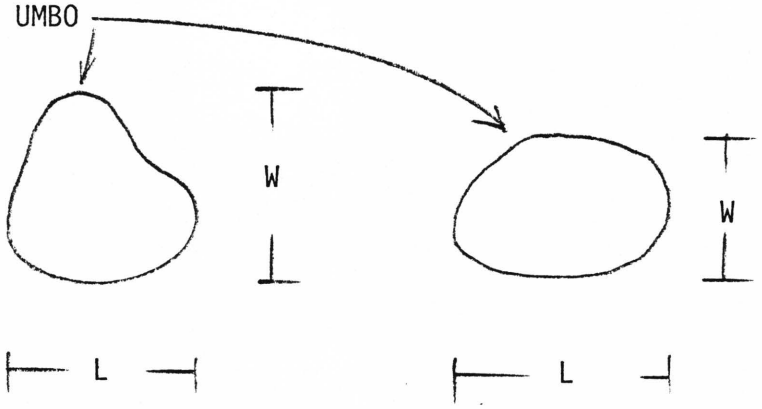
Figure 7. Sample of a page of larval measurements for oysters and clams.

OYSTER LARVAE

CLAM (Tapes) LARVAE

STRAIGHT HINGE or
"D"-SHAPED LARVAE

LARVAE WITH AN UMBO



(1) Microscope used and magnification with ocular micrometer (e.g., Wild 125X)

(2) State size of smallest unit in micrometer as calibrated on microscope (e.g., @ unit = 9.52μ)

(3) Record length and width in units (to nearest 1/10)

e.g.:	L	W
	9.8	8.7
	10.0	8.9
	9.4	8.7
	9.8	8.8
	10.0	9.0
	9.2	8.5
	9.5	8.6
	9.8	8.8
	9.7	8.7
	9.6	8.5
Average:	9.7	8.7

(4) Calculate average size in microns (i.e., multiply average # units by # microns in @ unit:

92μ x 83μ
(length x width)

pseudonana, Bellerochea polymorpha, and an unidentified Cryptophyte flagellate) gives the best results, but a mixture of two of those cultures gives satisfactory results (Sunderlin et al., 1976).

Prior to filtering the larval cultures, no quantitative measurements (cell counts or turbidities) are made on the algae remaining in the larval cultures. However, a pale green color is observed in the cultures, indicating that food is still present.

After the proper diet is distributed to the larval culture containers, the antibiotic is added. Streptomycin sulfate in liquid form is used primarily but other antibiotics are being tested in the hatchery. Vet-Strep, an aqueous form (manufactured by Merck & Co., Rahway, N.J.), with a concentration of 250 mg/cc, is used; however, powder Streptomycin sulfate is also acceptable. 0.2 ml Vet-Strep per liter of final larval culture is added; the resulting concentration of the antibiotic in the culture vessel is 50 mg/liter.

After the food and antibiotic(s) are added to the larval culture vessel, the larvae from the larval concentrate are added. The larvae are distributed equally among the culture vessels and the final volume in each vessel is reached by adding deep water.

The 15-, 30-, and 50-liter larval cultures are aerated with one air-line each—using a black rubber #12 stopper (or bung) as a weight on the air-line. Only slow, gentle

aeration is used. In the 379-liter cultures, two air-lines (with stoppers as weights) are used to keep the larvae in suspension.

Both techniques of keeping larvae in suspension work well when an initial stocking density of 10 larvae/ml is used. On each filtration day, the larvae are returned to the same size and number of containers. Therefore, when metamorphosis is reached, there are usually 5-6 Tapes larvae per ml. For oysters, the concentration is closer to 1/ml when setting size is reached.

Cleaning of Culture Vessels

It is preferable but not essential to have twice as many containers as one uses for any given experiment. This way, the extra clean containers can air dry (upside-down) for the days they are not being used. Air drying in a hatchery is one of the best ways to control contamination since most chemicals (Chlorox) can be harmful to larvae if misused. The 15-, 30-, and 50-liter containers are best scrubbed with one's clean hands and a thorough rinsing with deep water followed by draining (and preferably air drying) inverted or upside-down on a slatted surface to allow air circulation inside the buckets. A stiff brush—clean and only to be used for hatchery culture containers—may be used for scrubbing culture vessels, especially the 379-liter vessels.

A good rule of thumb for hatchery vessels/containers is: if found upside-down, vessel is clean; if found right

side-up, assume vessel to be dirty and scrub, rinse and drain before using.

IV. SETTING AND METAMORPHOSIS

Tapes japonica larvae are transferred into fiberglas setting flumes, 10 x 1.3 x 0.5 ft (3.05 x 0.38 x 0.15 m) when the average length is 200-225μ, usually Day 12-16. The larvae in the flumes are filtered every other day and batch-fed algal cultures for 4-8 days until the larvae have set and have completed metamorphosis. Four air-lines are placed in each flume. After the larvae have completed metamorphosis, the flumes are placed on continuous flow, receiving flod from 1 or more 2000-liter reactors.

After Day 30, when the juvenile population is evaluated for uniformity of size and survival, it is advisable to thin the population down to less than 75,000 per flume.

V. JUVENILE REARING

Data collection on spat is done every Tuesday morning. Tapes #20, #21, #22, and #23 are measured, according to the following procedure:

a) Collect entire population of batch #20 on a sieve. Rinse and spread out on towel to air-dry.

b) Weigh entire population to ±.1 g. Record data (see Table 2).

c) Take a sample of approximately .1 g. Weigh accurately to .1 mg. Count sample. Record weight and number. Repeat this step until at least 200 have been counted, or 10 samples taken.

d) Divide total weight of all samples counted by total count. Record data.

e) Take a sample which, by weight, should contain 10-20 clams. Weight = 10-20 x value calculated under step d).

f) Measure length and width of up to 16 clams sampled under step e). Record data.

g) Return batch #20 to flume. Repeat steps a) to g) on batches #21, #22, and #23.

TABLE 2. TAPES CLAM SPAT DATA

DATE_____ BATCH #___ TECHNICIAN_____

TOTAL POPULATION WEIGHT_____grams

SAMPLE	WEIGHT	NUMBER
1	_____	_____
2	_____	_____
3	_____	_____
4	_____	_____
5	_____	_____
6	_____	_____
7	_____	_____
8	_____	_____
9	_____	_____
10	_____	_____
TOTALS	W = _____	N = _____ W/N = _____

NUMBER	LENGTH	WIDTH	NUMBER	LENGTH	WIDTH
1	_____	_____	9	_____	_____
2	_____	_____	10	_____	_____
3	_____	_____	11	_____	_____
4	_____	_____	12	_____	_____
5	_____	_____	13	_____	_____
6	_____	_____	14	_____	_____
7	_____	_____	15	_____	_____
8	_____	_____	16	_____	_____

VI. LITERATURE CITED

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Loosanoff, V.L., H.C. Davis and P.E. Chanley, 1966. Dimensions and shapes of larvae of some marine bivalve mollusks. Malacologia 4(2):351-435.

Sunderlin, J.B., P.T. Baab and E.M. Patry, 1976. Growth of clam and oyster larvae on different algal diets in a tropical "artificial upwelling" mariculture system. Proc., World Mariculture Soc. (in press).

APPENDIX D

ARTIFICIAL UPWELLING MARICULTURE:

PHYTOPLANKTON COUNTING PROCEDURE

Phytoplankton Counting

Directions for the use of the

Speirs-Levy Eosinophil Counter:

Loading Counter

1. Mix sample thoroughly.
2. Take lens-shaped sample droplet with bacteriological transfer loop from mixed sample, while sample bottle is still in motion.
3. Touch loop to edge of one of the four chambers on the counter (see Fig. 1); sample should fill most of the chamber and at least cover the squares of the chamber.
4. Allow sample to settle while chamber is on microscope stage for about 10 minutes before counting, or until cells are all in the same focal plane.
5. Count two samples for each vessel or culture.

Counting Sample

1. Always try to count at least 200 cells total (will give approximately 15% expected error) in each of two duplicate samples.
2. The squares in each chanber (160 of them) are arranged in eight rows of 20 each. There are four chambers, so two cultures can be loaded at once on one counter. If a very dense culture is being counted, scatter the squares being counted over the entire area of the chamber (e.g., count every other row of squares, or just the top and bottom row). After counting, calculate the fraction of ruled area counted and express this as a portion of the entire ruled area "A" (see Fig. 1).

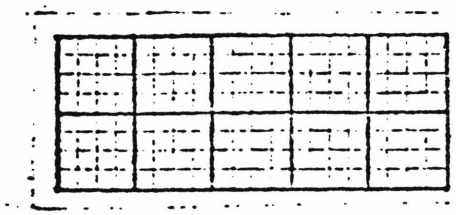


Figure 1

The area of the grid is "A".

A Method to Obtain Cell Count Reproducibility

We will consider replicate cell counts (counts taken for two samples from the same culture) sufficiently reproducible if they are not statistically significantly different. To determine statistical significant differences between replicate counts, we must compute a confidence interval.

1. Take the cell count for the half area of the counting chamber field as explained in the preceding section. Call this "count 1". The example in the preceding section was 110.

2. Substitute the half-area count for X in the following equations for computation of confidence interval limits.

   $$\text{Upper limit} = X + 2.42 + 1.96\sqrt{(X + 1.5)}$$

   $$\text{Lower limit} = X + 1.42 - 1.96\sqrt{(X + 0.5)}$$

   If X = 110, then we obtain:

   $$\text{Upper limit } 110 + 2.42 + 1.96\sqrt{(110 + 1.5)} = 133.12$$

   $$\text{Lower limit } 110 + 1.42 - 1.96\sqrt{(110 + 0.5)} = 90.82$$

The upper and lower limits so obtained define the boundaries of a span called the confidence interval. It is the range of values where the true count is expected to fall 95 times out of 100.

3. Take a second sample from the culture and obtain a half-area count. Call it "count 2."

4. Compute a confidence interval as for count 1.

5. Compare the confidence intervals for count 1 and count 2. If they overlap, then we will accept the counts as being reproducible. If the intervals do not overlap, then the counts are not reproducible and additional double samples should be taken until a reproducible count is obtained.

Two confidence intervals overlap when the upper limit of one interval falls above the lower limit of the other interval. For example, these confidence intervals overlap:

90.82 -- 133.12 and 99.90 -- 143.98

These confidence intervals do not overlap:

62.0 -- 99.0 and 99.90 -- 143.98

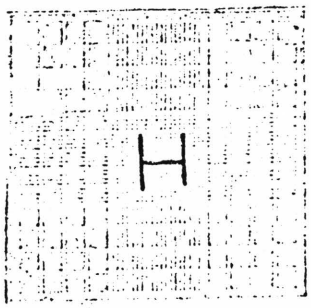
4.4.3.2 Phytoplankton Counting for Counts >10^6/Milliliter

1 - Load duplicate samples into the two chambers of a Spencer "Brightline" hemocytometer.

2 - In each chamber there are nine large squares, each 1 mm^2; the central large square (area "H" in diagram) is divided into 25 groups of sixteen small squares each.

3 - Count at least 100 cells in each duplicate sample, by counting the appropriate fraction of the central large square (see Table below).

4 - To calculate the cell concentration in cells per ml, multiply the sum of the duplicate counts by the appropriate factor from Table below.



(diagram)

Estimated density (cells/ml)	Fraction of central large square (1 mm^2) to count	Obtain cells/ml by multiplying sum of duplicates by:
1×10^6	1.0	0.5×10^4
2×10^6	4/5	0.625×10^4
3×10^6	3/5	0.833×10^4
4×10^6	2/5	1.25×10^4
$5\text{-}10 \times 10^6$	1/5	2.5×10^4

5 - Clean as described for the Speirs-Levy Chamber.

NOTE: When comparing confidence intervals for overlap, the upper limit of the interval computed for the lower cell count is compared to the lower limit of the interval computed for the higher count. If they overlap, the counts are sufficiently reproducible. In the examples, 90.82 -- 133.12 is the lower count confidence interval and 99.90 -- 143.98 is the higher count confidence interval. Since 133.12 falls above 99.90, we accepted the counts as reproducible.

Directions for Use of Hemocytometer

A hemocytometer (A.H. Thomas "Brightline") is used for counting dense (>10^6/ml) PT cultures, according to the instructions given in section 4.4.3.2.

APPENDIX E

ARTIFICIAL UPWELLING MARICULTURE:

AQUACULTURE BUDGET GENERATOR

PROGRAM LISTINGS

May 1977

(a) AQUA2B

(b) INDATA

(c) INDISK

(d) SUMDATA

```
GET ACP/AQUA23
*WORKFILE ACP/AQUA23: ALGOL, 658 RECORDS, SAVED
LIST
1000 BEGIN
2000 COMMENT
3000
4000              AQUACULTURE BUDGET GENERATOR PROGRAM
5000                          METRIC
5100
5200      VERSION 23, REVISED 5.27.76 BY G ALLEN
5300        ST CROIX ARTIFICIAL UPWELLING PROJECT APPLICATION
6000
7000 ;
8000 FILE      REMIN(KIND=REMOTE),
9000           REMOUT(KIND=REMOTE,MAXRECSIZE=14),
10000           LINEOUT(KIND=PRINTER);
10500 FILE DISKIN2(KIND=DISK,FILETYPE=7,TITLE="ACP/SUMDATA1.");
11000 FILE      DISKIN(KIND=DISK,FILETYPE=7,TITLE="ACP/BNAR.");
11500 FILE  DISKIN3(KIND=DISK,FILETYPE=7,TITLE="ACP/BNAR2.");
12000       ARRAY      VAR[0:960],A[0:10],C902[0:900],A903[0:900],A910[0:900];
12500      ARRAY SUMTIT[0:28,0:5],A911[0:900];
13000       ARRAY WT[0:900],TMP[0:5];
14000      INTEGER    I,TIME,IWANT,IREC,INDX,J,IPICK,LOW,HIGH,CAPITAL,II,
14050                  LENGTH,GRWPRP,IJ,K;
15000      REAL       LAG306,LAG807,LAG809,DUM,VAL,VALU;
15050 BOOLEAN TEST;
16000      LABEL      CALC925,NORITE,ENDOFPROG,START,CHANGES,LISTIT,INSERT,
17000                 PICKONE,MOREBNGES,C204,C207,C402,SECTION?,LIST1,ERR1,
17500                  LIST2,LIST3,TABLES,FX903,FX910,LSST,LAST;
18000 FORMAT  FMTOUT1  ("IF YOU WANT TO:  (1) CHANGE AN ASSUMPTION"
19000                   ,";(2) LIST A VARIABLE;"/T5,"(3) RUN THE "
20000                   ,"BUDGET PROGRAM;(4) CHANGE A TABLE;"
21000                  ,"(5) STOP THE PROGRAM."/T5,
21500                 "TYPE 1,2,3,4 OR 5"),
22000           BRIEF1("PICKONE. FOR HELP TYPE 9"),
24000          FMTOUT2    ("TYPE ASSUMPTION NUMBER, COMMA, AND NEW"
25000                       ," VALUE",/,"WHEN DONE TYPE '999,0'"),
27210           FMTOUT5("IF YOU WANT TO:"/,T5,"(1) SEE A SECTION"/
27220                   T5,"(2) SEE AN ASSUMPTION",/,T5,
27230                   "(3) SEE A TABLE",/,T5,
27240                   "(4) RETURN TO THE PROGRAM",/,
27250                   "TYPE 1,2,3, OR 4"),
27260           FMTOUT6("TYPE THE RANGE OF THE SECTION YOU WISH TO SEE",/,
27270                   "(E.G. 1,47)"),
27280          FMTOUT7("IF YOU WANT TO:",/,T10,"(1) SEE THE"
27290                  " MORTALITY ADJ. FACTOR TABLE"
27300                 ,/,T10,"(2) SEE THE COMPARTMENT SIZE TABLE",/,
27310                 "TYPE 1 OR 2"),
27320         FMTOUT8(5("T",J3,"=",F7.4,X1,)),
27340         FMTOUT9("TO SPECIFY THE TABLE:",/,
27350                 T10,"(1) A903",/,T10,"(2) A910",/,
27360                 T10,"(3) RETURN TO THE PROGRAM",/,
27370                 "TYPE 1,2 OR 3"),
27380        FMTOUT10("TYPE THE RANGE, COMMA, NEW VALUE"
27390                 ,/,"(E.G.5,12,0.02). WHEN DONE TYPE '999,999,999'"),
28000           FMTERR1    (I3,"  IS NOT A VALID NUMBER FOR "
29000                       "AN ASSUMPTION***TRY AGAIN"),
29500        FMTOUT4   ("TYPE THE ASSUMT. NUMBER YOU WISH TO SEE",/,
29510                   "IF YOU WISH TO SEE ANOTHER ASSUMT. - TYPE ",/,
```

```
GET ACP/AQUA23
#WORKFILE ACP/AQUA23: ALGOL, 658 RECORDS, SAVED
LIST
1000 BEGIN
2000 COMMENT
3000
4000              AQUACULTURE BUDGET GENERATOR PROGRAM
5000                          METRIC
5100
5200      VERSION 23, REVISED 5.27.76 BY G ALLEN
5300        ST CROIX ARTIFICIAL UPWELLING PROJECT APPLICATION
6000
7000 ;
8000 FILE      REMIN(KIND=REMOTE),
9000           REMOUT(KIND=REMOTE,MAXRECSIZE=14),
10000           LINEOUT(KIND=PRINTER);
10500 FILE DISKIN2(KIND=DISK,FILETYPE=7,TITLE="ACP/SUMDATA1.");
11000 FILE      DISKIN(KIND=DISK,FILETYPE=7,TITLE="ACP/BNAR.");
11500 FILE  DISKIN3(KIND=DISK,FILETYPE=7,TITLE="ACP/BNAR2.");
12000       ARRAY     VAR[0:960],A[0:10],C902[0:900],A903[0:900],A910[0:900];
12500      ARRAY SUMTIT[0:28,0:5],A911[0:900];
13000       ARRAY WT[0:900],TMP[0:5];
14000      INTEGER    I,TIME,IWANT,IREC,INDX,J,IPICK,LOW,HIGH,CAPITAL,II,
14050                  LENGTH,GRWPRP,IJ,K;
15000      REAL       LAG806,LAG807,LAG809,DUM,VAL,VALU;
15050 BOOLEAN TEST;
16000      LABEL      CALC925,NORITE,ENDOFPROG,START,CHANGES,LISTIT,INSERT,
17000                 PICKONE,MOREBNGES,C204,C207,C402,SECTION7,LIST1,ERR1,
17500                  LIST2,LIST3,TABLES,FX903,FX910,LSST,LAST;
18000 FORMAT  FMTOUT1  ("IF YOU WANT TO:  (1) CHANGE AN ASSUMPTION"
19000                   ,"/(2) LIST A VARIABLE"/T5,"(3) RUN THE "
20000                   ,"BUDGET PROGRAM/(4) CHANGE A TABLE"
21000                  ,"(5) STOP THE PROGRAM."/T5,
21500                 "TYPE 1,2,3,4 OR 5"),
22000           BRIEF1("PICKONE. FOR HELP TYPE 9"),
24000          FMTOUT2    ("TYPE ASSUMPTION NUMBER, COMMA, AND NEW"
25000                      ," VALUE",/,"WHEN DONE TYPE '999,0'"),
27210           FMTOUT5("IF YOU WANT TO:"/,T5,"(1) SEE A SECTION"/
27220                   T5,"(2) SEE AN ASSUMPTION",/,T5,
27230                   "(3) SEE A TABLE",/,T5,
27240                   "(4) RETURN TO THE PROGRAM",/,
27250                   "TYPE 1,2,3, OR 4"),
27260           FMTOUT6("TYPE THE RANGE OF THE SECTION YOU WISH TO SEE",/,
27270                   "(E.G. 1,47)"),
27280          FMTOUT7("IF YOU WANT TO:",/,T10,"(1) SEE THE"
27290                   " MORTALITY ADJ. FACTOR TABLE"
27300                  ,/,T10,"(2) SEE THE COMPARTMENT SIZE TABLE",/,
27310                  "TYPE 1 OR 2"),
27320         FMTOUT8(5("T",J3,"=",F7.4,X1,)),
27340         FMTOUT9("TO SPECIFY THE TABLE:",/,
27350                 T10,"(1) A903",/,T10,"(2) A910",/,
27360                 T10,"(3) RETURN TO THE PROGRAM",/,
27370                 "TYPE 1,2 OR 3"),
27380        FMTOUT10("TYPE THE RANGE, COMMA, NEW VALUE"
27390                 ,/,"(E.G.5,12,0.02). WHEN DONE TYPE '999,999,999'"),
28000           FMTERR1    (I3,"  IS NOT A VALID NUMBER FOR "
29000                       "AN ASSUMPTION***TRY AGAIN"),
29500        FMTOUT4   ("TYPE THE ASSUMT. NUMBER YOU WISH TO SEE",/,
29510                   "IF YOU WISH TO SEE ANOTHER ASSUMT. - TYPE ",/,
```

```
29520                  "IN THER NEXT ASSUMT. YOU WISH TO SEE. WHEN",/,
29530                  "DONE TYPE '-999'"),
29535          FMTOUT11(T22,"PHYSICAL PLANT REQUIREMENTS",/,T12,
29540                   "TOP LINE FOR SINGLE BATCH BOTTON LINE FOR",
29545                   " ONE BATCH PER MONTH",/,"MNTH",T7,"NUMBER",
29550                  T17,"SPACE",T24,"FLOWRATE",T35,"HEATING",
29555                  T44,"PUMPING",T54,"AERATION",T64,"FEED COST"),
30000            FMTOUT3  (I3,X1,E16.8,X2,8A6),
30100         FMTOUT12("TYPE TRANSFER NUMBER, AREA (M2), WEIGHT(G)",'
30150                  "(E.G. 3,0.02,150.0) WHEN DONE TYPE 999");
31000 SWITCH   SWLAB:=C204,C207,C402;
31500 SWITCH SWLAB1:=LIST1,LIST2,LIST3,PICKONE;
34000     VALUE ARRAY ASST(1,2,4,5,8,10,11,13,14,15,16,17,18,19,20,
35000                      22,24,26,27,29,31,34,35,36,47,101,102,
36000                      103,104,105,106,107,108,109,110,201,202,
37000                      203,204,205,206,207,208,209,210,211,212,213,
38000                      214,216,222,301,302,303,304,305,312,401,402,403,404,
39000                      405,406,407,408,409,410,411,412,413,414,415,416,
40000                  417,418,419,420,426,431,441,442,443,445,501,502,503,
41000                      504,505,506,507,509,601,602,603,604,605,
41025                      606,608,610,611,618,620,641,642,643,644,
42000                      701,703,704,705,801,802,803,
43000                      804,805,806,808,809,901,920,921,927,928);
44000     VALUE ARRAY CALC(204,207,402);
44100     VALUE ARRAY RITE(931,936,932,937,933,938,934,939,935,
44200                      940,941,807,947,615,950,949,944,946,
44300                      945,414,943,948);
44400   DEFINE TEMP=FOR I:=1 STEP 1 UNTIL 950 DO
44420          IF VAR[I] NEQ 0.0 THEN
44440          WRITE(REMOUT,<I3,E16.8>,I,VAR[I])#;
45000     DEFINE RITEOUT=A[0]:=VAL;
45500            VAR[IREC]:=VAL;
46000                      WRITE(DISKIN[IREC],11,A[*])#,
47000            REED=     READ(DISKIN[IREC+1],11,A[*])#;
47100   READ(DISKIN3[1],301,A903[*]);
47200   READ(DISKIN3[2],301,A910[*]);
47300    READ(DISKIN[3],301,A911[*]);
47400    FOR I:=0 STEP 1 UNTIL 300 DO BEGIN
47500       A903[I+300]:=A910[I];
47600       A903[I+600]:=A911[I];
47700    END;
48000     FOR I:=0 STEP 1 UNTIL 122 DO
49000          READ(DISKIN[ASST[I]], * ,VAR[ASST[I]]);
49100    FOR I:=0 STEP 1 UNTIL 25 DO
49110    READ(DISKIN2,<4A6>,FOR J:=0 STEP 1 UNTIL 3 DO SUMTIT[I,J]);
50000 PICKONE:      WRITE(RE
51000               READ(REMIN, / ,IWANT);
51500               CAPITAL:=0;
52000               IF IWANT EQL 1 THEN GO TO CHANGES ELSE
53000               IF IWANT EQL 2 THEN GO TO LISTIT ELSE
54000               IF IWANT EQL 3 THEN GO TO START ELSE
54500                IF IWANT EQL 4 THEN GO TO TABLES ELSE
54510                IF IWANT EQL 5 THEN GO TO ENDOFPROG ELSE
54517              IF IWANT EQL 9 THEN
54519              BEGIN
54521                WRITE(REMOUT,F
54523                GO TO PICKONE;
54525              END ELCE
54527              GO TO PICKONE;
```

```
54530 TABLES:  WRITE(REMOUT,FMTOUT9);
54540          READ(REMIN,/,IPICK);
54550          IF IPICK EQL 1 THEN
54560          BEGIN
54570               WRITE(REMOUT,FMTOUT10);
54580          FX903:  READ(REMIN,/,LOW,HIGVALU);
54584                IF LOW EQL 999 THEN GO TO PICKONE;
54592                FOR I:=LW STEP 1 UNTIL HIGH DO
54596                BEGIN
54600                  A903[I]:=VALU;
54608                END;
54610                  WRITE(DISKIN3[1],301,A903[*]);
54612                GO TO FX903;
54620          END ELSE
54630          IF IPICK EQL 2 THEN
54640          BEGIN
54650               WRITE(REMOUT,FMTOUT12);
54660           FX910:READ(REMIN,/,TIME);
54670                 IF TIME EQL 999 THEN GO TO PICKONE;
54680                 READ(REMIN,/,VALU,VAL);
54950               END ELSE
54960                 IF IPICK EQL 3 THEN GO TO PICKONE;
55000 CHANGES:       WRITE(REMOUT,FMTOUT2);
56000 MORECHANGES:   READ(REMI, / ,IREC,VALU);
57000                IF IREC EQL 999 THEN GO TO PICKONE;
57050          IF IREC EQL 808 THEN
57100          BEGIN
57150             VAL:=-LN(VALU)/180.0;
57200             FOR I:=1 STEP 1 UNTIL 901 DO
57250             A903[I-1]:=EX(-VAL/I);
57300          END;
57350          WRITE(DISKIN[3],901,A903[*]);
57500              READ(DISKIN[IREC],11,A[*]);
58000              IF MASKSEARC(IREC,4"FFF",ASST) NEQ -1 THEN
59000              BEGIN
60000              IF INDX:=MASKSEARCH(IREC,4"FFF",CALC) NEQ -1 THEN GO TO
61000              SWLAB[INDX+1];
61500              GO TO INSERT;
62000              C204: VALU:=1800*VALU**0.5;
63000                    GO TO INSERT;
64000              C207: VALU:=28000.0*VALU**.548;
65000                    GO TO INSERT;
67000              C402: VAL:=95000.0*VALU**.565;
68000                    RITEOUT;  REED;
69000                    VAL:=140000.0*VALU**.554;
70000                    RITEOUT;  REED;
71000                    VAL:=230000.0*VALU**.538;
72000                    RITEOUT;  REED;
73000                    VAL:=510000.0*VALU**.50;
74000                    RITEOUT;  REED;
75000                    VAL:=42000.0*VALU**.438;
76000                    RITEOUT;  REED;
77000                    VAL:=14.0*VALU**(-.169);
78000                    RITEOUT;  REED;
79000                    VAL:=86.0*VALU**(-.346);
80000                    RITEOUT;  REED;
81000                    VAL:=130.0*VALU**(-.262);
82000                    RITEOUT;  REED;
83000                    VALU:=150.0*VALU**(-.354);
```

```
84000                       READ(DISKIN[IREC],11,A[*]);
85000      INSERT:          A[0]:=VALU;
85500                       VAR[IREC]:=VALU;
86000                       WRITE(DISKIN[IREC],11,A[*]);
87000                       WRITE(REMOUT,<"##">);
88000                       GO TO MORECHANGES;
09000                  END;
90000                   WRITE(REMOUT,FMTERR1,IREC);
91000                   GO TO MORECHANGES;
91010 LISTIT:   WRITE(REMOUT,FMTOUT5);
91020           READ(REMIN,/,IPICK);
91030           GO TO SWLAB1[IPICK];
91040           GO TO LISTIT;
91050 LIST1:    WRITE(REMOUT,FMTOUT6);
91060           READ(REMIN,/,LOW,HIGH);
91070           FOR I:=LOW STEP 1 UNTIL HIGH DO
91072           BEGIN
91074             IF HIGH GTR 950 THEN HIGH:=950;
91076             IF VAR[I] NEQ 0 THEN
91080             WRITE(REMOUT,<I3,F30.10>,I,VAR[I]);
91084           END;
91090           GO TO LISTIT;
91500 LIST2:          WRITE(REMOUT,FMTOUT4);
92000  LSST: READ(REMIN,/,IREC);
93000                 BEGIN
93500                      IF IREC EQL -999 THEN GO TO LISTIT;
93600                      IF MASKSEARCH(IREC,4"FFF",ASST) EQL -1 THEN
93700                      BEGIN
93800                         WRITE(REMOUT,<I3," IS NOT A VALID ASST."
93850                                 " ***** TRY AGAIN">,IREC);
93870                         GO TO LSST;
93880                      END;
94000                      READ(DISKIN[IREC],11,A[*]);
95000                      WRITE(REMOUT,FMTOUT3,IREC, FOR I:=0 STEP 1
96000                      UNTIL 7 DO A[I]);
97000                 END;
97500                 GO TO LSST;
97510 LIST3:   WRITE(REMOUT,FMTOUT7);
97520          READ(REMIN,/,IPICK);
97530          IF IPICK EQL 1 THEN
97540          BEGIN
97550               WRITE(REMOUT,<"MORTALITY ADJ. FACTOR TABLE (A903)"
97555                    " ,%, IN 10 DAY INTERVALS"/>);
97560               FOR I:=1 STEP 5 UNTIL 150 DO
97570               WRITE(REMOUT,FMTOUT8,I,A903[I],I+1,A903[I+1],
97580                    I+2,A903[I+2],I+3,A903[I+3],I+4,A903[I+4]);
97590          END ELSE
97600          IF IPICK EQL 2 THEN
97610          BEGIN
97620              WRITE(REMOUT,<"AREA (M2)-WEIGHT (G) RELATIONS ", '
97630                   "(A910,A911) FOR CONTAINER SIZE TRANSFER"/>);
97640              FOR I:=0 STEP 1 UNTIL LENGTH - 1 DO
97650              WRITE(REMOUT,<X10,I3,2F12.4>,I+1,A910[I],A911[I]);
97660           END;
97670          GO TO LISTIT;
97700 START:
97705  TEST:=FALSE;
98100 % TIME OR WEIGHT DEPENDENT GROWTH LIMIT
98150  HIGH:=899;
```

```
98200 IF VAR[2] GTR 0 THEN HIGH:=MIN(VAR[2]-1.0,899);
98250 LOW:=0;
98300 %INITIALIZE VARIABLES
98305  FOR I:=457,458,459,460 DO VAR[I] := 0.0;
98310 FOR I:=311,435,437,452,453,454,455,456,463,514,612,924,925,926
98315   DO VAR[I] := 0.0;
98325  II:=0;
98350 WT[LOW]:=VAR[805];
98400 C902[LOW]:=VAR[901];
98450 VAR[922]:=HIGH+1;
98500 %  BIOLOGICAL SUBSYSTEM
98550 %    SET STARTING DAY (1-360)
98560   IJ:=VAR[10];
98565 %  STARTING WEIGHT,NUMBER OF TRAYS,SHARE OF TRANSFER
98570   VAR[98]:=VAR[805]*VAR[11];
98575   VAR[99]:=VAR[19]*1000.0/VAR[98];
98580   VAR[900]:=1.0;
98585   VAR[706]:=1.0;
98600 FOR I:=LOW STEP 1 UNTIL HIGH DO
98650 BEGIN
98660     IF I EQL HIGH THEN TEST:=TRUE;
98700 %  SET TEMPERATURE, AMBIENT AND OPERATING
98750    VAR[229]:=VAR[201];
98800    IF VAR[202] GTR 0 THEN VAR[229]:=VAR[202];
99050 %  OXYGEN CONSUMPTION (MG/HR)
99070    VAR[520]:=VAR[503]+VAR[620]*VAR[229];
99100    VAR[513]:=(VAR[520]*WT[I]**VAR[501])*C902[I];
99110 %  PHYTOPLANKTON PRODUCTION
99120 %  DEPTH FACTOR
99130   IF VAR[18] GTR VAR[22] THEN
99131     BEGIN
99133       WRITE(REMOUT,<"ACTUAL POOL DEPTH EXCEEDS COMPENSATIN~ DEPTH",
99135             2(X2,F8.1)>,VAR[18],VAR[22]);
99137       VAR[51]:=VAR[22];
99140     END ELSE VAR[51]:=VAR[18];
99150    VAR[51]:=VAR[51]-(VAR[51]**2)/(2.0*VAR[22]);
99160 %  EXPOSURE FACTOR
99170   VAR[52]:=VAR[24]/VAR[51];
99180 %  CONVERSION EFFICIENCY
99200    VAR[53]:=1.0+VAR[26]*VAR[52]+VAR[27]*VAR[52]**2.0;
99202   IF VAR[53] LSS 0.0 THEN BEGIN
99204   WRITE(REMOUT,<"CALCULATED PHYTOPLANKTON CONVERSION EFFICIENCY IS",
99206         " NEGATIVE,",/,"IMPLYING EXCESSIVE FLOWRATE. COMPUTATION ",
99208         "TERMINATED. REDUCE VARIABLE 24">);
99210   GO TO PICKNE;
99212   END;
99214 %  RATE OF PROTEIN PRODUCTION (G/CM2/DAY)
99220   VAR[54]:=6.25*(VAR[20]+VAR[644]);
99230   VAR[55]:=VAR[54]*VAR[53]*VAR[24];
99300 %  GROWTH INCREMENT
99350    WT[I+1]:=WT[I];
99380 %  ANIMAL GROWTH FUNCTION
99400    BOBIU:=1 STEP 1 UNTIL 2 DO
99500 %  FEEDING CRITERION
99650 % LIMITED BY FEED QUANTITY AND TYPE
99655   VAR[56]:=VAR[29]*VAR[31];
99661 % CONVERSION EFFICIENCY
99667   VAR[57]:=VAR[34]+VAR[35]*LN(VAR[56])+VAR[36]*LN(VAR[56])**2;
```

```
99670 %  CONVERSION FACTOR
99673   VAR[58]:=VAR[14]*VAR[15]*VAR[17];
99676 %  RATE OF FEEDING PHYTO (G/SEC/ANIMAL IN)
99685   VAR[59]:=VAR[56]*WT[I]**VAR[802];
99691 %  GROWTH INCREMENT PER DAY
99694   VAR[807]:=VAR[58]*VAR[57]*VAR[59]*86400.0;
99700 % LIMITED BY METABOLITE/OXYGEN CONC/PH NOT OP
99750 % LIMITED BY SPACE, NOT OP
99900         WT[I+1]:=WT[I+1]+VAR[807];
99950     END;
99975       VAR[807] := (WT[I+1] - WT[I])/2.0;
100000   WT[I+1]:=(WT[I+1]+WT[I])/2.0;
100010 %  FOOD CONSUMPTION, CORRECTED FOR MORTALIT  G/SEC/ANIMAL IN
100020   VAR[621]:=VAR[59]*C902[I];
100031 %  METABOLITE PRODUCTION (G/DAY) AMMONIUM-NH3 (TH MAX)
100032    VAR[432]:=(1.0-VAR[57])*VAR[621]/6.25;
100034 % NH3 DISSOCIATION BASED ON HENDERSON-HASSELBACH EQUATION AND
100035 % LOG KA=.2804+2717.(1/ABCTEMP) PKA=VAL
100036    VAL:=.2804+2717.0/(VAR[229]+273.0);
100037 % PROPORTION OF NH3 (VALU) THEN ACTUAL NH3
100038     VALU:=1.0/(10.0**(VAL-VAR[445])+1);
100039    VAR[436]:=VAR[432]*VALU;
100040     VAR[463]:=VAR[621]*VAR[420]*VAR[603];
100050 % TARGET WEIGHT REACHED?
100100    IF WT[I+1] LSS VAR[920] THEN
100150    BEGIN
100200       IF I EQL 899 THEN
100250       BEGIN
100300         WRITE(REMOUT,<"TARGET WEIGHT CANNOT BE REACHED IN ",
100350              "900 DAYS",/,"RESET ASSUMPTIONS JUST CHANGED">);
100400         GO TO PICKONE;
100450       END;
100500    END ELSE
100550    BEGIN
100600       VAR[922]:=I+1;
100650         TEST:=TRUE;
100700    END;
100850 % SURVIVAL
100900     C902[I+1]:=A903[I+1]*VAR[901];
100950 % SURVIVAL MODIFIED BY O2/METAB CONC, PH, SPACE, FEED, NOT OP
100960 % WEIGHT & NUMBER, SINGLE BATCH
100970     ` VAR[915]:=WT[I+1]*C902[I+1];
100985 VAR[916]:=C902[I+1];
100990 %  TRANSFER CRITERION
100992    IF VAR[915] GEQ VAR[98] THEN
100994    BEGIN
100996 %  SHARE OF TRANSFER & NUMBER OF TRAYS (/TRAY INPUT)
100999       IJ:=IJ-1;
101000       IF IJ GTR 0 THEN BEGIN
101001         VAR[900]:=VAR[900]*VAR[11];
101002         VAR[706]:=VAR[706]+VAR[900];
101003         VAR[98]:=VAR[98]*VAR[11];
101006       END ELSE
101008       BEGIN
101010         VAR[922]:=I+1;
101012         TEST:=TRUE;
101014         I:=HIGH;
101016       END;
101018    END;
```

```
101020 %  SINGLE OR REPEATED BATCH - VAR[1] IS 0 OR 1
101050     IF VAR[1] EQL 1 THEN
101100     BEGIN
101150       IF TEST THEN GO TO LAST ELSE
101200        IF (I+1)/VAR[806]-INTEGERT((I+1)/VAR[806]) EQL 0 THEN
101250        BEGIN
101300 LAST:
101350 % CUMULATED FEED (G/SEC/ANIMAL IN)
101400          VAR[612]:=*+VAR[621];
101450 % CUMULATED OXYGEN CONSUMPTION (MG/HR)
101500          VAR[514]:=*+VAR[513];
101550 % CUMULATED METABOLITE PRODUCTION
101600          VAR[435]:=*+VAR[432];
101650          VAR[437]:=*+VAR[436];
101670           VAR[462]:=*+VAR[463];
101700 % CUMULATED SPACE REQ, PER INPUT
101750           VAR[924]:=*+VAR[900];
101800 % WEIGHT AND NUMBER OF ANIMALS (G OR #/ANIMAL IN)
101850          VAR[925]:=*+WT[I+1]*C902[I+1];
101900          VAR[926]:=*+C902[I+1];
101910 %  CUMULATE NUMBER OF TRANSFERS
101920          VAR[706]:=VAR[706]+VAR[900];
101950        END;
102050     END;
102075          IF WT[I+1]  GEQ VAR[920] THEN I:= HIGH;
102100 END;
102120 %  SHARE OF TRANSFER AND NUMBER OF TRAYS (/ANIMAL IN)
102130     VAR[900]:=VAR[900]/VAR[99];
102140     VAR[706]:=VAR[706]/VAR[99];
102150     VAR[924]:=VAR[924]/VAR[99];
102200 % TRANSFER VALUES
102250 IF VAR[1] EQL 1 THEN
102300 BEGIN
102325     VAR[915]:=VAR[925];
102350     VAR[621]:=VAR[612];
102400     VAR[513]:=VAR[514];
102450     VAR[432]:=VAR[435];
102500     VAR[436]:=VAR[437];
102550     VAR[900]:=VAR[924];
102600     VAR[916]:=VAR[926];
102620      VAR[463]:=VAR[462];
102650 END;
102655 %  TOTAL NUMBER OF ANIMALS STARTED
102660      VAR[926]:=1000.0*VAR[921]/(WT[VAR[922]]*C902[VAR[922]]);
102665  %  TOTAL FEEDRATE, AREA (UTILIZED & TOTAL) & FLOW N PHYTO TANKS
102670      VAR[640]:=VAR[926]*VAR[621];
102675      VAR[661]:=VAR[640]/VAR[55];
102680      VAR[662]:=VAR[661]*(1.0+VAR[16]);
102685      VAR[663]:=VAR[661]*VAR[24];
102690      VAR[664]:=VAR[661]*VAR[18]/1000000.0;
102695      VAR[#29]:=VAR[601]*VAR[664];
100700S%EMTOTAL WEIGHT OF ANI
102705      VAR[915]:=VAR[915]*VAR[926];
102710 %  DESIGN METABOLITE LOADING (NH3) G/CM3
102750 VAR[440]:=VAR[441]*VAR[442]/10.0**7.0;
102800 % FLOWRATE THROUGH TANKS  CM3/SEC
102805 %  RECIRCULATION PROPORTION
102806  VAR[493]:=VAR[663]/VAR[915]+VAR[443];
```

```
102808     IF VAR[416] GTR 0.0 THEN
102811     BEGIN
102817       VAR[494]:=1.0-VAR[493]/VAR[416];
102820       IF VAR[494] LSS 0.0 THEN
102823       BEGIN
102826ESS THANWRITE(REMOUT,<"SPECIFIED FLOWRATE IN ANI
102829            " INFLOW. ",/,"RATE OF ",E16.8," (CM3/SEC/G OF ANIMAL ",
102832            "IS ASSUMED.">,VAR[493]);
102835         VAR[494]:=0.0;
102838       END;
102841     END ELSE VAR[494]:=VAR[414];
102844 %  CALCULATED FLOWRATE REQUIRED (CM3/SEC)
102850 VAL:=VAR[440]-VAR[4]/10.0**6.0;
102875 VALU:=(VAR[643]/100.0)*VAR[440]/VAL;
102900 VAR[438]:=(VAR[436]/VAL)/(1.0-VAR[494]+VAR[494]*VALU);
102950     VAR[438]:=VAR[438]*VAR[926]/VAR[915];
102955 TEST:=FALSE;
102965 IF VAR[416] GTR 0.0 THEN BEGIN
102975     IF VAR[416] LSS VAR[438] THEN TEST:=TRUE;
102985     VAR[438]:=VAR[416]*(1.0-VAR[494]);
102990   END ELSE IF VAR[495]:=VAR[493]*(1.0-VAR[494]) LSS VAR[438] THEN BEGIN
102995      TEST:=TRUE;
102998      VAR[438]:=VAR[495];
103000   END;
103005   IF TEST THEN
103010 WRITE(REMOUT,<"WARNING - FLOWRATE IN ANIMAL TANKS INSUFFICIENT TO ",
103020     "MEET DESIGN ",/,"METABOLITE LOADING. MODIFY ONE OF VAR 4,414,",
103030     "416,441,442,443,603.">);
103040 VAR[438]:=VAR[438]*VAR[915]*86.4;
103100 % AERATION REQUIREMENT FLOWRATE
103150 % TOTAL OXYGEN CONSUMPTION
103200 VAR[517]:=VAR[513]*VAR[926];
103250 % OXYGEN INTAKE CONC (OMEGAF)  MG/L
103300 VAR[515]:=11.52*EXP((-0.0207)*VAR[201]);
103350 % CALCULATED FLOWRATE  L/DAY
103400 VAL:=VAR[502]-VAR[515];
103500 VAR[515]:=11.52*EXP((-0.0207)*VAR[229]);
103550 VALU:=((-VAR[5]))*(VAR[515]-VAR[502])/VAL;
103600 VAR[439]:=VAR[517]+VAL*VAR[438]*(1.0-VAR[494]
103620              +VAR[494]*VALU)/24.0;
103650 % DIRECT OXYGENATION, IF NECESSARY
103700 IF VAR[439] GTR 0 THEN
103750 VAR[519]:=VAR[439]/(1000000.0*VAR[504])
103800 ELSE VAR[519]:=0.0;
105300 COMMENT    HEATING REQUIREMENTS, HEAT RECIRCULATING WATER
105400            THE AMOUNT OF TEMP LOSS (VAR[214]), HEAT INTAKE
105500            WATER TO OPERATE TEMPERATURE + 50% OF TEMP LSS
105600 ;
105700            VAR[200]:=((1.0-VAR[494])*(VAR[229]+0.5*VAR[214]-
105800                       VAR[201]) + VAR[494]*VAR[214]);
105900            VAR[215]:=VAR[200]*VAR[438];
106100 %  NUMBER OF TRAYS
106200       VAR[950]:=VAR[900]*VAR[926];
106300       VAR[916]:=VAR[916]*VAR[926];
106400
106550  % PUMP CAPACITY, INTAKE AND RECIRC (KW) - 100% EFFIC
106600     VAR[326]:=0.00001*VAR[663]*VAR[302];
106650    VAR[327]:=0.00001*VAR[443]*VAR[302]*VAR[915];
```

```
106700  VAR[306]:=VAR[312]*(VAR[19]*1000.0)**2.0;
106720  VAR[306]:=VAR[306]*VAR[950]/1000.0;
106810 %  FEEDING EQUIPMENT REQUIREMENT
106815  IF VAR[618] GTR 0.0 THEN BEGIN
106820           VAR[629]:=VAR[950]*VAR[605];
106840           VAR[627]:=VAR[618]*(INTEGER(VAR[900]/VAR[606])+1)*
106841                          (1.0+VAR[604])*VAR[401]/2000.0;
106850  END;
107000          FOR II:=438,215,438,235 DO VAR[II]:=VAR[II]/1000.0;
107100            IF CAPITAL EQL 1 THEN WRITE(REMOUT,<I2,7F10.1,/,X2,
107200            7F10.1>,J,VAR[916],VAR[950],VAR[438],VAR[215],VAR[326],
107300            VAR[518],VAR[627],VAR[926],VAR[924],VAR[438],VAR[235],
107400            VAR[306],VAR[519],VAR[607]);
107450          FOR II:=438,215,438,235 DO VAR[II]:=VAR[II]*1000.0;
117000 COM    (SECTION 2)
118000                        COST OF SPACE
119000 ;
119500 %  COST OF LAND
120000                VAR[111]:= VAR[101]/VAR[102];
121000                VAR[112]:= VAR[111]*VAR[928];
121100 % COST OF PHYTO TANKS
121200   VAR[124]:=(3.0*VAR[803]+1.0)*SQRT(VAR[662])/100.0;
121300   VAR[125]:=4.0*VAR[18]/100.0+2.0*(VAR[18]/100.0)**2.0;
121400   VAR[126]:=VAR[804]*VAR[124]*VAR[125]*10000.0/VAR[662];
121500   VAR[127]:=VAR[126]*VAR[928];
122000                VAR[113]:=VAR[103]*(VAR[927]/((VAR[927]+1.0)
122500                          **VAR[104]-1.0)+VAR[928]);
123000 %  COST OF ANIMAL TANKS
124000                VAR[115]:=VAR[105]*(VAR[927]/((VAR[927]+1.0)
124500                          **VAR[106]-1.0)+VAR[928]);
125000                VAR[116]:= VAR[641]*VAR[107];
126000          VAR[117]:=VAR[116]*(VAR[927]/((VAR[927]+1.0)
126500                     **VAR[642]-1.0)+VAR[928]);
127000                VAR[118]:=VAR[108]*(VAR[927]/((VAR[927]+1.0)
127500                          **VAR[109]-1.0)+VAR[928]);
8000                 VAR[119]:= VAR[111]+VAR[103]+VAR[105]+VAR[116]+VAR[126];
129000                VAR[120]:= VAR[112]+VAR[113]+VAR[115]+VAR[117]+VAR[127];
130000          VAR[121]:=(VAR[111]+VAR[105]+VAR[116])/VAR[110]+VAR[108];
130500          VAR[122]:=(VAR[112]+VAR[115]+VAR[117])/VAR[110]+VAR[118];
131000 CMMENT              END OF COST SPACE                              ;
161000                COMMENT                                          (SECTION 9)
162000                                                                 COST OF FED
163000                ;
175000  IF VAR[602] GTR 0.0 THEN BEGIN
175050        -         VAR[615]:=VAR[621]*30.0/(WT[VAR[922]]
175060                          *C902[VAR[922]]);
175090                VAR[609]:=VAR[607]*(VAR[927]/((VAR[927]+1.0)
175095                          **VAR[608]-1.0)+VAR[928]);
175100  VAR[613]:=VAR[629]*30.0*VAR[611]/(VAR[921]*VAR[610]);
175110               VAR[614]:= VAR[609]/(12.0*VAR[921])+VAR[613];
175120               VAR[616]:= VAR[615]*VAR[602];
175130                 VAR[617]:=VAR[616]+VAR[614]*1000.0/WT[VAR[922]];
175500  END;
176000 COMMENT             END OF SECTION 4&5                            ;
177000 COMMENT             (SECTION 6)
178000                     COST OF WASTE TREATMENT
179000 ;
179050 % DISCHARGE WASTE TREATMENT
179100    VAR[444]:=VAR[621]*VAR[926]*VAR[603]*86400;
```

```
179200 VAR[433]:=VAR[419]*VAR[444];
179300     IF VAR[432]:=VAR[431]*VAR[915]
179400      GTR VAR[433] THEN VAR[433]:=VAR[432];
179800 VAR[432]:=VAR[444]*VAR[420];
179900 VAR[422]:=VAR[494]*VAR[438];
180000   VAR[421]:=VAR[432]*VAR[417]/((VAR[438]-VAR[422])*100.0);
180020   VAR[423]:=(VAR[432]-VAR[433])/VAR[421];
180040   IF VAR[423] GTR VAR[438]-VAR[422] THEN
180060   BEGIN
180080     VAR[423]:=VAR[438]-VAR[422];
180100     WRITE(REMOUT,<"WARNING - DISCHARGE WASTE TREATMENT TOO ",
180120        "INEFFICIENT TO MEET",/,"EPA REGULATIONS IN SINGLE PASS">);
180140   END;
180210  IF VAR[423] GTR 0 THEN
180220     BEGIN
180300 VAR[452]:=(18.243*VAR[423]**0.565)*VAR[402];
180400 VAR[453]:=(31.758*VAR[423]**0.554)*VAR[403];
180800 VAR[457]:=(181.063*VAR[423]**(-0.169))*VAR[402];
180900 VAR[458]:=(16237.723*VAR[423]**(-0.346))*VAR[403];
180910     END;
181200   VAR[424]:=VAR[452]+VAR[453];
185000     VAR[425]:=(VAR[424]*VAR[401])/2000.0;
186000     VAR[427]:=VAR[425]*(VAR[927]/((VAR[927]+1.0)
186500               **VAR[426]-1.0)+VAR[928]);
187000     VAR[428]:=VAR[427]*1000.0/(365.0*(VAR[438]-VAR[422]));
188000 VAR[429]:=(VAR[457]+VAR[458]+VAR[411]*
188100           VAR[412]*VAR[413]*VAR[422]/1000000.0)/VAR[438]*1000.0;
190000     VAR[430]:=VAR[428]+VAR[429];
191000 COMMENT             END OF SECTION 6                          ;
192000 COMMENT             (SECTION 7)
193000                     COST OF AERATION
194000 ;
194500  SECTION7:
195000     VAR[508]:=VAR[506]*(VAR[519]*VAR[401])/2000.0;
196000     VAR[510]:=VAR[508]*(VAR[927]/((VAR[927]+1.0)
196500               **VAR[509]-1.0)+VAR[928]);
197000     VAR[511]:=(VAR[519]*VAR[413]*24.0*365.0+VAR[507]*VAR[508]/100.0);
199000     VAR[512]:=VAR[510]+VAR[511];
200000 COMMENT             END OF SECTION 7                          ;
201000 COMMENT             (SECTION 8)
202000                     COST OF HEAT
203000 ;
205000   IF VAR[202] GTR 0.0 THEN BEGIN
206000 VAR[217]:=(VAR[229]-VAR[201]-0.5*VAR[214])*(VAR[438]-VAR[422])*
206010           (1.0-EXP(VAR[203]*VAR[216]));
207000     VAR[218]:=5900.0*VAR[216]**0.5;
208000     VR[219]:=VAR[218]*VAR[401]/2000.0;
209000     VAR[220]:=VAR[219]*((VAR[206]/100.0)+(VAR[927]/((VAR[927]
209500               +1.0)**VAR[205]-1.0)**VAR[205]-1.0)+VAR[928]);
210000     VAR[221]:=VAR[215]-VAR[217];
211000     VAR[223]:=(1.0+VAR[222]/100.0)*(VAR[221]/24.0)*(1.0/VAR[212]);
212000     VAR[224]:=30.7*VAR[223]**0.548;
213000     VAR[225]:=VAR[224]*VAR[401]/2000.0;
214000     VAR[226]:=VAR[225]*(VAR[927]/((VAR[927]+1.0)
214500               **VAR[208]-1.0)+VAR[928]);
215000     VAR[227]:=VAR[225]*VAR[209]/100.0+VAR[210]*VAR[211]*365.0+VAR[223]
216000               *24.0*365.0*VAR[213]/(1000000.0);
217000     VAR[228]:=VAR[220]+VAR[226]+VAR[227];
218000   END;
```

```
220000 COMMENT             END OF SECTION 8                              ;
220010 COMMENT                   SECTION 10
220020                         COST OF PUMPING
220030 ;
220032   VAR[330]:=0.0;VAR[331]:=0.0;
220035   VAR[328]:=VAR[306]/VAR[8];
220040  FOR I:=326 STEP 1 UNTIL 329 DO BEGIN
220045    VAR[I]:=VAR[I]/VAR[301];
220050 VAR[307]:=(560.0*VAR[I]**0.6)*(1.0+VAR[303]/100.0)
220055            *VAR[401]/2000.0;
220060 VAR[308]:=VAR[307]*(VAR[927]/((VAR[927]+1.0)**VAR[304]
220065            -1.0) + VAR[928]);
220070 VAR[309]:=VAR[307]*VAR[305]/100.0 + VAR[I]*8760.0*
220080              VAR[413];
220090   VAR[I-6]:=VAR[308]+VAR[309];
220092   VAR[330]:=*+VAR[307];
220094   VAR[3#1]:=*+VR[#08];
220098 END;
220100     VAR[311]:=VAR[320]+VAR[321];
220105    VAR[310]:=VAR[322]*VAR[8];
220110 COMMENT               END OF SECTION 10
220120 ;
220130 COMMENT                  (SECTION 11)
220140                  COST OF LABOR - HARVEST,TRANSFER, AND CLEANING
220150 ;
220240           VAR[706]:=(VAR[706]*VAR[611])/(VAR[703]);
220500  VAR[708]:=VAR[661]*VAR[611]*VAR[704]*12.0/(VAR[705]*10000.0);
221000 COMMENT              (SECTION 9)
222000                      COST SUMMARY
223000 ;
224000   VAL:=VAR[921]*365.0/VAR[806];
225000   VAR[931]:=VAR[122]*VAR[950]/VAL;
226000   VAR[932]:=VAR[120]*VAR[662]/(10000.0*VAL);
227000   VAR[933]:=VAR[228]/VAL;
228000   VAR[934]:=VAR[323]/VAL;
229000   VAR[935]:=VAR[512]/VAL;
230000   VAR[936]:=VAR[708]/VAL;
231000   VAR[937]:=(VAR[311]+VAR[663]*0.864*365.0*VAR[13]+VAR[663]*86.4
231100              *365.0*VAR[664]*VAR[610])/VAL;
232000   VAR[938]:=VAR[662]/10000.0;
233000   VAR[939]:=VAR[310]/VAL;
234000   VAR[940]:=VAR[18]/100.0;
235000   VAR[941]:=VAR[616];
236000   VAR[942]:=VAR[663]/10.0**6.0;
237000   VAR[943]:=VAR[932]+VAR[934]+VAR[936];
238000   VAR[944]:=VAR[53];
239000   VAR[945]:=VAR[430]*(VAR[433]-VAR[422])*0.365/VAL;
240000   VAR[946]:=WT[VAR[922]];
241000   VAR[947]:=VAR[706]*VAR[926]*365.0/(VAR[806]*VAL);
242000   VAR[948]:=VAR[921];
243000   VAR[949]:=VAR[701];
245000   VAR[951]:=VAR[47]*1000.0/(WT[VAR[922]]*C902[VAR[922]]);
246000   VAR[952]:=VAR[57];
247000   VAR[953]:=0.0;
248000   FOR I:=931 STEP 2 UNTIL 951 DO
249000     VAR[953]:=*+VAR[I];
250000   VAR[954]:=VAR[922];
250100   VAR[955]:=(VAR[119]*VAR[662]/10000.0+VAR[121]*VAR[950]+VAR[219]
250200              +VAR[225]+VAR[330]+VAR[425]+VAR[508]+VAR[607])/1000.0;
```

```
250300    VAR[956]:=(VAR[932]*VAL+VAR[122]*VAR[950]+VAR[220]+VAR[226]
250400                +VAR[331]+VAR[427]+VAR[510]+VAR[609])/1000.0;
251000    WRITE(REMOUT,<T30,"OUTPUT SUMMARY">);
252000    WRITE(REMOUT,<T2,"ANIMAL PRODUCTION COST($/KG OUTPUT)",
E50E80URES">);T45,"PERFOR
252300          FOR I:=931 STEP 2 UNTIL 955 DO BEGIN
252350    IJ:=I-931;
252400     WRITE(REMOUT,<2(F12.4,X1,4A6)>,VAR[I],
252500       FOR J:=0 STEP 1 UNTIL 3 DO SUMTIT[IJ,J],VAR[I+1],
252600       FOR J:=0 STEP 1 UNTIL 3 DO SUMTIT[IJ+1,J]);
252700    END;
258000 GO TO PICKONE;
259000 ENDOFPROG:
260000 END.
#
REMOVE
#
```

```
GET ACP/INDATA
#WORKFILE ACP/INDATA: DATA, 123 RECORDS, SAVED
LIST
100    1              1.00000 FOR SINGLE BATCH, 1 FOR REPEATED
200    2            900.0    TIME LIMIT ON GROWTH (DAYS) MAX 900
300    4              0.0000INLET NH3 CONC (MG-N/L)
400    5              0.90000OXYGENATION EFFIC IN RECIRC LOOP (PROPN)
500    8              1.0000NUMBER OF RECIRCULATION PUMPS IN ANIM TANKS
600   10             5.0     NUMBER  OF TRANSFER CYCLES
700   11             4.0     TRANSFER DECISION: FINAL WT/START WT
800   13              0.00   COST OF DEEP SEA WATER ($/M3)
900   14             2.65    TOTAL ANIM WEIGHT/WET MEAT WT (#)
1000  15               2.50  TOTAL MEAT/MEAT PROTEIN (#,D.M. BASIS)
1100  16               0.00  RESERVE PHYTO TANK CAPY (PROPN)
1200  17               5.00  WET WEIGHT/DRY WEIGHT (#)
1300  18             100.0   PHYTO POOL DEPTH (CM)
1400  19             20.0    MAXIMUM WT OF ANIMALS  (KG/TRAY)
1500  20    0.000000448      NUTRIENT CONCENTRATION DSW (G-N/CM3)
1600  22             400.0   COMPENSATION DEPTH FOR PHYTO (CM)
1700(C)24            0.00106 FLOWRATE TO PHYTO TANK (C
1800  26               0.0   COEFF B1 IN PHYTO EFFIC FN (#)
1900  27    -1750000000.0    COEFF B2 IN PHYTO EFFIC FN (#)
2000  29               3.0   FEED RATE/BIOL EFFIC FD RATE (#)
2100  31    0.000000011808   BIOL EFFIC SPECIFIC FD CRITERION (G/SEC/1G AN)
2200  34   -16.89145         COEFF A0 IN ANIMAL EFFIC FN (#)
2300  35     -1.891173        COEFF A1 IN ANIM EFFIC FN   (#)
2400  36     -0.0518          COEFF A2 IN ANIMAL EFFIC FN (#)
2500  47              0.0010COST OF "SPAT" ($/HEAD)
2600 101            10000.0000COST OF LAND ($/HA)
2700 102            8000.0000AREA UTILIZABLE BY BUILDING OR STRUCTURE (M2/HA)
2800 103              10.0000CAPITAL COST OF PHYTO TANKS INCL  ($/M2)
2900 104               5.0000LIFE OF PHYTOPLANKTO TANKS (YR)
3000 105               0.0000COST OF COVER (PLASTIC WITH STEEL OR WOOD FRAME) ($/

3100 106               5.0000LIFE OF COVER (YR)
3200 107               0.0063OFFICE & STOR AGE REQUIRED AS PROPORTION OF TANK
3300 108              20.0000CAPITAL COST OF ANIMAL TANKS ($/TRAY)
3400 109               5.0000LIFE OF TANK COMPARTMENTS (YR)
3500 110               3.0   STACKING FACTOR PER AREA STRUCTURE (TRAYS/M2)
3600 201              20.0000MEAN AMBIENT SEAWATER TEMPERATURE (°C)
3700 202               0.0000OPERATING TEMPERATURE OF TANKS (°C)
3800 203               0.5000EFFICIENCY OF HEAT EXCHANGER (PROPN)
3900 204               0.0000CAPITAL COST FUNCTION=HEAT EXCHANGER WHERE C IS CAPI

4000 205              10.0000LIFE OF HEAT EXCHANGER (YR)
4100 206               2.0000ANNUAL MAINTENANCE CHARGE (% OF CAP)
4200 207               0.0000CAPITAL COST FUNCTION=GAS OR OIL FIRED HEATERS
4300 208              10.0000LIFE OF HEATER (YR)
4400 209               3.0000ANNUAL MAINTENANCE (% OF CAP)
4500 210               4.0000LABR REQUIREMENT FOR HEATER (HR/DAY)
4600 211               8.0000MAINTENANCE ENGINEER WAGE RATE ($/HR)
4700 212               0.9000EFFICIENCY OF HEATER (PROPN)
4800 213              11.0000COST OF FUEL ($/MIL BTU)
4900 214               0.0000LOSS OF HEAT WITHIN SYSTEM (°C)
5000 216               0.0000AREA OF HEAT EXCHANGER (M2)
5100 222              50.0000RESERVE CAPACITY OF HEATER REQUIRED  (%)
5200 301               0.8500EFFICIENC OF PUMP (PROPN)
5300 302              10.0000PUMP LIFT -INTKE (M)
5400 303             100.0000STANDBY REQUIREMENT (PERCENT)
```

```
5500 304            10.0000LIFE OF PUMP (YEARS)
5600 305             3.0000PUMP MAINTENANCE (PERCENT)
5700 312         0.00000025RECIRC PUMPING POWER COEFF (#)
5800 401         2400.0000ENGINEERING NEWS RECORD COST OF CONSTRUCTION INDEX(E

5900 402             1.0000CAPITAL COST FUNCTION FOR SCREENING
6000 403             1.0000CAPITAL COST FUNCTION FOR FILTRATION
6100 404             0.0000CAPITAL COST FUNCTION FOR NITRIFICATION
6200 405             0.0000CAPITAL COST FUNCTION FOR CARBON ABSORPTION
6300 406             0.0000CAPITAL COST FUNCTION FOR OZONATION
6400 407             0.00000 & M COST OF SCREENING
6500 408             0.00000 & M COST OF FILTRATION
6600 409             0.00000 & M COST OF NITRIFICATION
6700 410             0.00000 & M COST OF CARBON ABSORPTION
6800 411             5.00000ZONE DOSAGE
6900 412            22.0000ELECTRICAL CONCUMPTION IN OZONE PRODUCTION (KWH/KG)
7000 413             0.0400ELECTRICAL POWER COST ($/KWH)
7100 414             0.9000PROP OF FLOW IN ANIMAL TANKS RECIRC (#)
7200 415             0.1000PROPORTION OF RECIRCULATION TREATED WITH CARBON
7300 416             0.0000FLOWRATE THROUGH TANKS (CM3/SEC/G)
7400 417            90.0000EFFIC OF WASTE TRT SYSTEM IN REM SUSP SOL(%)
7500 418            90.0000EFFIC OF WASTE TRT SYSTEM IN REM NITRATE(%)
7600 419             0.1200EPA DISCHARGE REQUIREMENT (G SS/G FD/DAY)
7700 420             0.8000WASTE PRODUCTION (G SS/G FD(DM BASIS))
7800 426            10.0000LIFE OF WASTE TREATMENT PLANT (YR)
7900 431             0.0030ALTERN EPA DISCHARGE REQ (G SUSP SOL/G ANIM)
8000 441            10.0000DESIGN AMMONIACONCENTRATION (% OF 96 HR LC50)
8100 442             1.2000ACUTE TOXIC AMMONIA CONC. (MG/L)
8200 443             0.0000DIRECT SW FLOWRATE TO ANIM TANKS (CM3/SEC/G
8300 445             8.0   OPERATING PH
8400 501             0.88000XYGEN CONSUMPTION RATE (DELTA)
8500 502             5.0000MINIMUM TOLERABLE O2 CONCENTRATION IN TANK (MG/L)
8600 503        -0.013908  COEFF 30 IN SPECIFIC O2 CONSN FN (#)
8700 504             0.6000AERATION EFFICIENCY, SURFACE AERATOR (PROPN)
8800 505             1.0000ELECTRIC MOTOREFFICIENCY (PROPN)
8900 506           500.0000CAPITAL COST OF AIR LIFT PUMP ($/INST KW)
9000 507             1.0000MAINTENANCE COT OF AERATOR (% OF CAP)
9100 509             5.0000LIFE OF AERATOR (YR)
9200 601             0.0025PHYTO POOL RECIRC POWER REQ (KW/M3)
9300 602             0.0000COST OF ARTIFIC FEED (0 IF NONE) ($/KG-PROT)
9400 603             2.5000PHYTO COMPOSITION(G-DM/G-PROT)
9500 604             0.0000FEEDING UNITS STANDBY CAPACITY (PROP)
9600 605             0.0000FREQ OF FEEDING ARTIF FOOD (TIMES/DAY)
9700 606             0.0000CAPACITY OF FEEDING EQUIP (M2/DAY/UNIT)
9800 608             0.0000LIFE OF FEEDING EQUIP  (YEAR)
9900 610             0.0000COST  OF ADDED NUTRIENT ($/KG-N)
10000 611             4.00  WAGE RATE ($/HR)
10100 618             0.0000CAPITAL COST OF FEEDING EQUIP ($/UNIT)
10200 620         0.002413 COEFF 31 IN SPECIFIC O2 CONSN FN (#)
10300 641           200.00  CAPITAL COST OF OFFICE,STORAGE,&C ($/M2)
10400 642            15.00  LIFE OF OFFICE,STORAGE,&C (YEAR)
10500 643             0.0000EFFIC OF RECIRC WASTE TRT IN REM NH3 (%)
10600 644             0.0   NUTRIENT ADDED TO INTAKE WATER(G-N/CM3)
10700 701             0.1000COST OF LABOR=HARVEST SUPERVISORY ($/KG OUTPUT)
10800 703            20.0000 RATE OF TRANSFER AND HARVEST (TRAYS/HR)
10900 704             1.0000FREQUENCY OF HAND CLEANING PHYTO TANK (TIMES/MONTH)
```

```
 11000 705                50.0000RATE OF HAND CLEANING PHYTO TANKS (M2/HR)
 11100 801                 7.2000"BIOLOGICAL ZERO" TEMPERATURE (°C)
 11200 802                 0.6700COEFFICIENT   ALPHA (METABOLIC BODY SIZE)
 11300 803                 3.0000NUMBER OF PHYTOPLANKTON TANKS
 11400 8 4                 1.0000COST OF EXCAVATION,PHYTO TANK ($/M3)
 11500 805               0.01025 INITIAL WEIGHT (G)
 11600 806                 5.00  BATCH INTERVAL (DAYS)
 11700 808                 0.80  PROP ANIMALS SURVIVING AFTER 180 DAYS (#)
 11800 809                 0.0000NOT OP
 11900 901                 1.0000STARTING POPU LATION, 4TH STAGE, T=0
 12000 920                10.0000MAX WEIGHT OF ANIMAL AT HARVEST (G)
A1BT00K02BATCH)          5000.0000TARGET OUTPUT FRO
 12200 927                 0.1000INTEREST RATE ON SINKING FUND (PROPORTION)
 12300 928                 0.1000RETURN ON     CAPITAL (PROPORTION)
 #
 REMOVE
 #
```

```
GET ACP/INDISK
#WORKFILE ACP/INDISK: ALGOL, 83 RECORDS, SAVED
LIST
1000 BEGIN
2000 FILE      DISKIN     (KIND=DISK,FILETYPE=7,TITLE="ACP/INDATA."),
2500           REMOUT(KIND=REMOTE),
5000           DISOUT    (KIND=DISK,MAXRECSIZE=14,BLOCKSIZE=420,
6000                      AREASIZE=30,AREAS=35,SAVEFACTOR=999,
7000                      TITLE="ACP/3NAR.",
7100         DISKOUT2(KIND=DISK,MAXRECSIZE=301,BLOCKSIZE=903,
7200                  AREASIZE=9,AREAS=2,SAVEFACTOR=999,
7300                  TITLE="ACP/3NAR2.");
8000 ARRAY    A[0:13],A903[0:900],A910[0:300],A911[0:300];
8100      VALUE ARRAY ASST(1,2,4,5,8,10,11,13,14,15,16,17,18,19,20,
8105                       22,24,26,27,29,31,34,35,36,47,101,102,
8110                       103,104,105,106,107,108,109,110,201,202,
8115                       203,204,205,206,207,208,209,210,211,212,213,214,
8120                       216,222,301,302,303,304,305,312,401,402,403,404,
8125                       405,406,407,408,409,410,411,412,413,414,415,
8130                       416,417,418,419,420,426,431,441,442,443,445,501,502,
8135                       503,504,505,506,507,509,601,602,603,604,
8140                       605,606,608,610,611,618,
8142                       620,641,642,643,644,701,703,801,802,803,
8145                       804,805,806,808,809,901,920,921,927,928);
8150     VALUE ARRAY CALC 204,207,402);
9000 INTEGER  I,IREC,ISET;
10000 LABEL    [illegible],INSERT,C204,C207,C402;
12000 SWITCH      SWLAB:=C204,C207,C402;
12100 DEFINE RITEOUT=  A[0]:=VAL;
12110                  WRITE(DISKIN[IREC],11,A[*])#,
12120        REED    =  READ(DISKIN[IREC],11,A[*])#;
12130     FILL A910[*] WITH 150(0);
12134 %  SURVIVAL FUNCTION, S=VAR[808] WHEN I = 180
12136      VALU:=-(LN(0.8)/180.0);
12140    FOR I:=1 STEP 1 UNTIL 301 DO BEGIN
12141             A903[I-1]:=EXP(-VALU*I);
12142      A903[I-1]:=EXP(-VALU*I);
12144      A910[I-1]:=EXP(-VALU*(I+299));
12146      A911[I-1]:=EXP(-VALU*(I+599));
12148    END;
12150      WRITE(DISOUT2[1],301,A903[*]);
12160      WRITE(DISKOUT2[2],301,A910[*]);
12170      WRITE(DISKOUT[3],301,A911[*]);
13000 AGAIN:    READ(DISKIN,<I3,F20.2,9A6>,IREC,FOR I:=0
14000           STEP 1 UNTIL 9 DO A[I])[EOF];
15000           VALU:=A[0];
15500          GO TO INSERT;
16000           IF ISET:=MASKSEARCH(IREC,4"FFF",ASST) NEQ -1 THEN
17000           BEGIN
18000                IF ISET:=MASKSEARCH(IREC,4"FFF",CALC) NEQ -1 THEN
18500                BEGIN
21000                GO TO SWLAB[ISET+1];
22000                C204: VALU:=1800*VALU**0.5;
23000                      GO TO INSERT;
24000                C207: VALU:=28000.0*VALU**.548;
24500                      RITEOUT;  REED;
25000                C402: VAL:=95000.0*VALU**.565;
26000                      RITEOUT;  REED;
```

```
27000                       VAL:=140000.0*VALU**.554;
28000                       RITEOUT;  REED;
29000                       VAL:=230000.0*VALU**.538;
30000                       RITEOUT;  REED;
31000                       VAL:=510000.0*VALU**.50;
32000                       RITEOUT;  REED;
33000                       VAL:=420000.0*VALU**.438;
34000                       RITEOUT;  REED;
35000                       VAL:=14.0*VALU**(-.169);
36000                       RITEOUT;  REED;
37000                       VAL:=86.0*VALU**(-.346);
38000                       RITEOUT;  REED;
39000                       VAL:=130.0*VALU**(-.262);
40000                       RITEOUT;  REED;
41000                       VALU:=150.0*VALU**(-.354);
42000                 INSERT:A[0]:=VALU;
43000                       WRITE(DISKOUT[IREC], * ,FOR I:=0
44000                       STEP 1 UNTIL 10 DO A[I]);
45000                 GO TO AGAIN;
46000                 END;
47000                 WRITE(DISKOUT[IREC],*,FOR I:=0 STEP 1 UNTIL 10 DO A[I]);
48000                 GO TO AGAIN;
49000            END;
49500             WRITE(REMOUT,<I3,"IS NOT A VALID ASSUMPTION #">);
49550             GO TO AGAIN;
50000 EF:      LOCK(DISKIN); LOCK(DISKOUT); LOCK(DISKOUT2);
51000 END.
#
REMOVE
#
```

```
GET ACP/SUMDATA
#NO FILE:ACP/SUMDATA.
GET ACP/SUMDATA1
#WORKFILE ACP/SUMDATA1: DATA, 26 RECORDS, SAVED
LIST
100 SPACE
200 PHYTO SPACE COST ($/KG)
300 HEAT
400        RECIRC COST ($/KG)
500 AERATION
600        LABOR COST ($/KG)
700 INTAKE WATER (PUMPED)
800 PHYTO TANKS AREA (M2)
900 RECIRCULATION PUMPING
1000  -"-  TANKS DEPT@ (M)
1100 FEED:ARTIFICIAL
1200  -"-  TANKS FLOW(M3/SEC)
1300      :PHYTOPLANKTON
1400  -"-  CONVERSION EFFIC
1500 WASTE TREATMENT
1600 ANIMAL INDIVID WEIGHT(G)
1700 LABOR,REGULAR
1800  -"-   BATCH WEIGHT (KG)
1900  -"- ,SUPERVISORY,&C
2000  -"-   NUMBER OF TRAYS
2100 LARVAE
2200  -"-   CONVERSION EFFIC
2300   TOTAL
2400  -"-   DAYS TO HARVEST
2500  TOTAL CAPITAL (000)
2600  ANN'L TOTAL CAP (000)
#
REMOVE
#
```

APPENDIX F

ARTIFICIAL UPWELLING MARICULTURE:

SAMPLE OUTPUT FROM BUDGET GENERATOR PROGRAM

(INFLUENCE OF POOL DEPTH

AND TURNOVER RATE ON PRODUCTION COST)

```
TYPE ASSUMPTION NUMBER, COMMA, AND NEW VALUE
WHEN DONE TYPE '999,0'
27,-1340000000.0
**
24,0.00133333
**
18,200
**
24,0.0016
**
999,0
```

OUTPUT SUMMARY

ANIMAL PRODUCTION COST ($/KG OUTPUT)		PERFORMANCE MEASURES		
0.0841	SPACE	0.2748	PHYTO	SPACE COST ($/KG)
0.0000	HEAT	0.2046		RECIRC COST ($/KG)
0.0032	AERATION	0.0874		LABOR COST ($/KG)
0.0686	INTAKE WATER (PUMPED)	33247.0172	PHYTO	TANKS AREA (M2)
0.5472	RECIRCULATION PUMPING	2.0000	-"-	TANKS DEPTH (M)
0.0000	FEED:ARTIFICIAL	0.5320	-"-	TANKS FLOW (M3/SEC)
0.5668	:PHYTOPLANKTON	0.8475	-"-	CONVERSION EFFIC
0.0524	WASTE TREATMENT	10.0292	ANIMAL	INDIVID WEIGHT (G)
0.2462	LABOR,REGULAR	5000.0000	-"-	BATCH WEIGHT (KG)
0.1000	-"- ,SUPERVISORY,&C	5714.4768	-"-	NUMBER OF TRAYS
0.1262	LARVAE	0.3073	-"-	CONVERSION EFFIC
1.7946	TOTAL	189.0000	-"-	DAYS TO HARVEST
699.9030	TOTAL CAPITAL (000)	153.2176	ANN'L	TOTAL CAP (000)

```
TYPE ASSUMPTION NUMBER, COMMA, AND NEW VALUE
WHEN DONE TYPE '999,0'
24,0.00187
**
999,0
```

OUTPUT SUMMARY

ANIMAL PRODUCTION COST ($/KG OUTPUT)		PERFORMANCE MEASURES		
0.0841	SPACE	0.2520	PHYTO	SPACE COST ($/KG)
0.0000	HEAT	0.1879		RECIRC COST ($/KG)
0.0032	AERATION	0.0801		LABOR COST ($/KG)
0.0732	INTAKE WATER (PUMPED)	30451.4127	PHYTO	TANKS AREA (M2)
0.5472	RECIRCULATION PUMPING	2.0000	-"-	TANKS DEPTH (M)
0.0000	FEED:ARTIFICIAL	0.5694	-"-	TANKS FLOW (M3/SEC)
0.5200	:PHYTOPLANKTON	0.7917	-"-	CONVERSION EFFIC
0.0524	WASTE TREATMENT	10.0292	ANIMAL	INDIVID WEIGHT (G)
0.2462	LABOR,REGULAR	5000.0000	-"-	BATCH WEIGHT (KG)
0.1000	-"- ,SUPERVISORY,&C	5714.4768	-"-	NUMBER OF TRAYS
0.1262	LARVAE	0.3073	-"-	CONVERSION EFFIC
1.7525	TOTAL	189.0000	-"-	DAYS TO HARVEST
662.7118	TOTAL CAPITAL (000)	144.7479	ANN'L	TOTAL CAP (000)

```
TYPE ASSUMPTION NUMBER, COMMA, AND NEW VALUE
WHEN DONE TYPE '999,0'
24,0.00213
**
999,0
```

```
                                  OUTPUT SUMMARY
 ANIMAL PRODUCTION COST($/KG OUTPUT)          PERFORMANCE MEASURES
      0.0841 SPACE                          0.2402 PHYTO SPACE COST ($/KG)
      0.0000 HEAT                           0.1793       RECIRC COST ($/KG)
      0.0032 AERATION                       0.0763       LABOR COST ($/KG)
      0.0791 INTAKE WATER (PUMPED)      29003.2620 PHYTO TANKS AREA (M2)
      0.5472 RECIRCULATION PUMPING          2.0000  -"-  TANKS DEPTH (M)
      0.0000 FEED:ARTIFICIAL                0.6178  -"-  TANKS FLOW(M3/SEC)
      0.4957      :PHYTOPLANKTON            0.7200  -"-  CONVERSION EFFIC
      0.0524 WASTE TREATMENT               10.0292 ANIMAL INDIVID WEIGHT(G)
      0.2462 LABOR,REGULAR               5000.0000  -"-   BATCH WEIGHT (KG)
      0.1000  -"- ,SUPERVISORY,&C        5714.4768  -"-   NUMBER OF TRAYS
      0.1262 LARVAE                         0.3073  -"-   CONVERSION EFFIC
      1.7341   TOTAL                      189.0000  -"-   DAYS TO HARVEST
    643.8918  TOTAL CAPITAL (000)         140.4345  ANN'L TOTAL CAP (000)
```

```
TYPE ASSUMPTION NUMBER, COMMA, AND NEW VALUE
WHEN DONE TYPE '999,0'
24,0.0024
**
999,0
```

```
                                  OUTPUT SUMMARY
 ANIMAL PRODUCTION COST($/KG OUTPUT)          PERFORMANCE MEASURES
      0.0841 SPACE                          0.2368 PHYTO SPACE COST ($/KG)
      0.0000 HEAT                           0.1768       RECIRC COST ($/KG)
      0.0032 AERATION                       0.0752       LABOR COST ($/KG)
      0.0874 INTAKE WATER (PUMPED)      28594.4381 PHYTO TANKS AREA (M2)
      0.5472 RECIRCULATION PUMPING          2.0000  -"-  TANKS DEPTH (M)
      0.0000 FEED:ARTIFICIAL                0.6863  -"-  TANKS FLOW(M3/SEC)
      0.4889      :PHYTOPLANKTON            0.6570  -"-  CONVERSION EFFIC
      0.0524 WASTE TREATMENT               10.0292 ANIMAL INDIVID WEIGHT(G)
      0.2462 LABOR,REGULAR               5000.0000  -"-   BATCH WEIGHT (KG)
      0.1000  -"- ,SUPERVISORY,&C        5714.4768  -"-   NUMBER OF TRAYS
      0.1262 LARVAE                         0.3073  -"-   CONVERSION EFFIC
      1.7356   TOTAL                      189.0000  -"-   DAYS TO HARVEST
    639.4812  TOTAL CAPITAL (000)         139.3638  ANN'L TOTAL CAP (000)
```

TYPE ASSUMPTION NUMBER, COMMA, AND NEW VALUE
WHEN DONE TYPE '999,0'
18,300
***
999,0

OUTPUT SUMMARY

ANIMAL PRODUCTION COST ($/KG OUTPUT)		PERFORMANCE MEASURES		
0.0841	SPACE	0.2059	PHYTO	SPACE COST ($/KG)
0.0000	HEAT	0.2216		RECIRC COST ($/KG)
0.0032	AERATION	0.0633		LABOR COST ($/KG)
0.0742	INTAKE WATER (PUMPED)	24069.8266	PHYTO	TANKS AREA (M2)
0.5472	RECIRCULATION PUMPING	3.0000	-"-	TANKS DEPTH (M)
0.0000	FEED:ARTIFICIAL	0.5777	-"-	TANKS FLOW (M3/SEC)
0.4907	:PHYTOPLANKTON	0.7805	-"-	CONVERSION EFFIC
0.0524	WASTE TREATMENT	10.0292	ANIMAL	INDIVID WEIGHT (G)
0.2462	LABOR,REGULAR	5000.0000	-"-	BATCH WEIGHT (KG)
0.1000	-"- ,SUPERVISORY,&C	5714.4768	-"-	NUMBER OF TRAYS
0.1262	LARVAE	0.3073	-"-	CONVERSION EFFIC
1.7242	TOTAL	189.0000	-"-	DAYS TO HARVEST
604.8969	TOTAL CAPITAL (000)	128.4736	ANN'L	TOTAL CAP (000)

TYPE ASSUMPTION NUMBER, COMMA, AND NEW VALUE
WHEN DONE TYPE '999,0'
24,0.00267
***
999,0

OUTPUT SUMMARY

ANIMAL PRODUCTION COST ($/KG OUTPUT)		PERFORMANCE MEASURES		
0.0841	SPACE	0.1986	PHYTO	SPACE COST ($/KG)
0.0000	HEAT	0.2137		RECIRC COST ($/KG)
0.0032	AERATION	0.0610		LABOR COST ($/KG)
0.0792	INTAKE WATER (PUMPED)	23185.8635	PHYTO	TANKS AREA (M2)
0.5472	RECIRCULATION PUMPING	3.0000	-"-	TANKS DEPTH (M)
0.0000	FEED:ARTIFICIAL	0.6191	-"-	TANKS FLOW (M3/SEC)
0.4732	:PHYTOPLANKTON	0.7283	-"-	CONVERSION EFFIC
0.0524	WASTE TREATMENT	10.0292	ANIMAL	INDIVID WEIGHT (G)
0.2462	LABOR,REGULAR	5000.0000	-"-	BATCH WEIGHT (KG)
0.1000	-"- ,SUPERVISORY,&C	5714.4768	-"-	NUMBER OF TRAYS
0.1262	LARVAE	0.3073	-"-	CONVERSION EFFIC
1.7117	TOTAL	189.0000	-"-	DAYS TO HARVEST
592.9490	TOTAL CAPITAL (000)	125.7941	ANN'L	TOTAL CAP (000)

```
TYPE ASSUMPTION NUMBER, COMMA, AND NEW VALUE
WHEN DONE TYPE '999,0'
24,0.00293
**
999,0
```

OUTPUT SUMMARY

ANIMAL PRODUCTION COST ($/KG OUTPUT)			PERFORMANCE MEASURES	
0.0841	SPACE	0.1959	PHYTO	SPACE COST ($/KG)
0.0000	HEAT	0.2109		RECIRC COST ($/KG)
0.0032	AERATION	0.0602		LABOR COST ($/KG)
0.0854	INTAKE WATER (PUMPED)	22871.2501	PHYTO	TANKS AREA (M2)
0.5472	RECIRCULATION PUMPING	3.0000	-"-	TANKS DEPTH (M)
0.0000	FEED:ARTIFICIAL	0.6701	-"-	TANKS FLOW (M3/SEC)
0.4670	:PHYTOPLANKTON	0.6728	-"-	CONVERSION EFFIC
0.0524	WASTE TREATMENT	10.0292	ANIMAL	INDIVID WEIGHT (G)
0.2462	LABOR,REGULAR	5000.0000	-"-	BATCH WEIGHT (KG)
0.1000	-"- ,SUPERVISORY,&C	5714.4768	-"-	NUMBER OF TRAYS
0.1262	LARVAE	0.3073	-"-	CONVERSION EFFIC
1.7117	TOTAL	189.0000	-"-	DAYS TO HARVEST
589.2928	TOTAL CAPITAL (000)	124.9378	ANN'L	TOTAL CAP (000)

```
TYPE ASSUMPTION NUMBER, COMMA, AND NEW VALUE
WHEN DONE TYPE '999,0'
24,0.00213
**
999,0
```

OUTPUT SUMMARY

ANIMAL PRODUCTION COST ($/KG OUTPUT)			PERFORMANCE MEASURES	
0.0841	SPACE	0.2185	PHYTO	SPACE COST ($/KG)
0.0000	HEAT	0.2351		RECIRC COST ($/KG)
0.0032	AERATION	0.0673		LABOR COST ($/KG)
0.0702	INTAKE WATER (PUMPED)	25592.2228	PHYTO	TANKS AREA (M2)
0.5472	RECIRCULATION PUMPING	3.0000	-"-	TANKS DEPTH (M)
0.0000	FEED:ARTIFICIAL	0.5451	-"-	TANKS FLOW (M3/SEC)
0.5209	:PHYTOPLANKTON	0.8271	-"-	CONVERSION EFFIC
0.0524	WASTE TREATMENT	10.0292	ANIMAL	INDIVID WEIGHT (G)
0.2462	LABOR,REGULAR	5000.0000	-"-	BATCH WEIGHT (KG)
0.1000	-"- ,SUPERVISORY,&C	5714.4768	-"-	NUMBER OF TRAYS
0.1262	LARVAE	0.3073	-"-	CONVERSION EFFIC
1.7504	TOTAL	189.0000	-"-	DAYS TO HARVEST
626.0680	TOTAL CAPITAL (000)	133.1872	ANN'L	TOTAL CAP (000)

```
TYPE ASSUMPTION NUMBER, COMMA, AND NEW VALUE
WHEN DONE TYPE '999,0'
18,350
**
999,0
```

OUTPUT SUMMARY

ANIMAL PRODUCTION COST ($/KG OUTPUT)		PERFORMANCE MEASURES		
0.0841	SPACE	0.2181	PHYTO	SPACE COST ($/KG)
0.0000	HEAT	0.2680		RECIRC COST ($/KG)
0.0032	AERATION	0.0660		LABOR COST ($/KG)
0.0689	INTAKE WATER (PUMPED)	25104.2343	PHYTO	TANKS AREA (M2)
0.5472	RECIRCULATION PUMPING	3.5000	-"-	TANKS DEPTH (M)
0.0000	FEED:ARTIFICIAL	0.5347	-"-	TANKS FLOW (M3/SEC)
0.5521	:PHYTOPLANKTON	0.8432	-"-	CONVERSION EFFIC
0.0524	WASTE TREATMENT	10.0292	ANIMAL	INDIVID WEIGHT (G)
0.2462	LABOR,REGULAR	5000.0000	-"-	BATCH WEIGHT (KG)
0.1000	-"- ,SUPERVISORY,&C	5714.4768	-"-	NUMBER OF TRAYS
0.1262	LARVAE	0.3073	-"-	CONVERSION EFFIC
1.7804	TOTAL	189.0000	-"-	DAYS TO HARVEST
635.7113	TOTAL CAPITAL (000)	133.5048	ANN'L	TOTAL CAP (000)

```
TYPE ASSUMPTION NUMBER, COMMA, AND NEW VALUE
WHEN DONE TYPE '999,0'
24,0,0024
**
999,0
```

```
TYPE ASSUMPTION NUMBER, COMMA, AND NEW VALUE
WHEN DONE TYPE '999,0'
24,0.0024
**
999,0
```

OUTPUT SUMMARY

ANIMAL PRODUCTION COST ($/KG OUTPUT)		PERFORMANCE MEASURES		
0.0841	SPACE	0.2044	PHYTO	SPACE COST ($/KG)
0.0000	HEAT	0.2509		RECIRC COST ($/KG)
0.0032	AERATION	0.0617		LABOR COST ($/KG)
0.0724	INTAKE WATER (PUMPED)	23456.3707	PHYTO	TANKS AREA (M2)
0.5472	RECIRCULATION PUMPING	3.5000	-"-	TANKS DEPTH (M)
0.0000	FEED:ARTIFICIAL	0.5630	-"-	TANKS FLOW (M3/SEC)
0.5169	:PHYTOPLANKTON	0.8009	-"-	CONVERSION EFFIC
0.0524	WASTE TREATMENT	10.0292	ANIMAL	INDIVID WEIGHT (G)
0.2462	LABOR,REGULAR	5000.0000	-"-	BATCH WEIGHT (KG)
0.1000	-"- ,SUPERVISORY,&C	5714.4768	-"-	NUMBER OF TRAYS
0.1262	LARVAE	0.3073	-"-	CONVERSION EFFIC
1.7486	TOTAL	189.0000	-"-	DAYS TO HARVEST
612.0632	TOTAL CAPITAL (000)	128.3128	ANN'L	TOTAL CAP (000)

```
TYPE ASSUMPTION NUMBER, COMMA, AND NEW VALUE
WHEN DONE TYPE '999,0'
24,0.00267
**
999,0
```

OUTPUT SUMMARY

ANIMAL PRODUCTION COST ($/KG OUTPUT)		PERFORMANCE MEASURES		
0.0841	SPACE	0.1956	PHYTO	SPACE COST ($/KG)
0.0000	HEAT	0.2400		RECIRC COST ($/KG)
0.0032	AERATION	0.0589		LABOR COST ($/KG)
0.0767	INTAKE WATER (PUMPED)	22408.5666	PHYTO	TANKS AREA (M2)
0.5472	RECIRCULATION PUMPING	3.5000	-"-	TANKS DEPTH (M)
0.0000	FEED:ARTIFICIAL	0.5383	-"-	TANKS FLOW (M3/SEC)
0.4945	:PHYTOPLANKTON	0.7535	-"-	CONVERSION EFFIC
0.0524	WASTE TREATMENT	10.0292	ANIMAL	INDIVID WEIGHT (G)
0.2462	LABOR,REGULAR	5000.0000	-"-	BATCH WEIGHT (KG)
0.1000	-"- ,SUPERVISORY,&C	5714.4768	-"-	NUMBER OF TRAYS
0.1262	LARVAE	0.3073	-"-	CONVERSION EFFIC
1.7305	TOTAL	189.0000	-"-	DAYS TO HARVEST
597.2646	TOTAL CAPITAL (000)	125.0527	ANN'L	TOTAL CAP (000)

TYPE ASSUMPTION NUMBER, COMMA, AND NEW VALUE
WHEN DONE TYPE '999,0'
24,0.00293
***
999,0

OUTPUT SUMMARY

ANIMAL PRODUCTION COST ($/KG OUTPUT)		PERFORMANCE MEASURES		
0.0841	SPACE	0.1912	PHYTO	SPACE COST ($/KG)
0.0000	HEAT	0.2346		RECIRC COST ($/KG)
0.0032	AERATION	0.0576		LABOR COST ($/KG)
0.0819	INTAKE WATER (PUMPED)	21881.8050	PHYTO	TANKS AREA (M2)
0.5472	RECIRCULATION PUMPING	3.5000	-"-	TANKS DEPTH (M)
0.0000	FEED:ARTIFICIAL	0.6411	-"-	TANKS FLOW (M3/SEC)
0.4833	:PHYTOPLANKTON	0.7032	-"-	CONVERSION EFFIC
0.0524	WASTE TREATMENT	10.0292	ANIMAL	INDIVID WEIGHT (G)
0.2462	LABOR,REGULAR	5000.0000	-"-	BATCH WEIGHT (KG)
0.1000	-"- ,SUPERVISORY,&C	5714.4768	-"-	NUMBER OF TRAYS
0.1262	LARVAE	0.3073	-"-	CONVERSION EFFIC
1.7245	TOTAL	189.0000	-"-	DAYS TO HARVEST
590.2257	TOTAL CAPITAL (000)	123.4796	ANN'L	TOTAL CAP (000)

TYPE ASSUMPTION NUMBER, COMMA, AND NEW VALUE
WHEN DONE TYPE '999,0'
24,0.0032
***
999,0

OUTPUT SUMMARY

ANIMAL PRODUCTION COST ($/KG OUTPUT)		PERFORMANCE MEASURES		
0.0841	SPACE	0.1906	PHYTO	SPACE COST ($/KG)
0.0000	HEAT	0.2338		RECIRC COST ($/KG)
0.0032	AERATION	0.0574		LABOR COST ($/KG)
0.0888	INTAKE WATER (PUMPED)	21810.2374	PHYTO	TANKS AREA (M2)
0.5472	RECIRCULATION PUMPING	3.5000	-"-	TANKS DEPTH (M)
0.0000	FEED:ARTIFICIAL	0.6979	-"-	TANKS FLOW (M3/SEC)
0.4817	:PHYTOPLANKTON	0.6460	-"-	CONVERSION EFFIC
0.0524	WASTE TREATMENT	10.0292	ANIMAL	INDIVID WEIGHT (G)
0.2462	LABOR,REGULAR	5000.0000	-"-	BATCH WEIGHT (KG)
0.1000	-"- ,SUPERVISORY,&C	5714.4768	-"-	NUMBER OF TRAYS
0.1262	LARVAE	0.3073	-"-	CONVERSION EFFIC
1.7299	TOTAL	189.0000	-"-	DAYS TO HARVEST
590.1087	TOTAL CAPITAL (000)	123.4026	ANN'L	TOTAL CAP (000)

```
TYPE ASSUMPTION NUMBER, COMMA, AND NEW VALUE
WHEN DONE TYPE '999,0'
18,100
***
24,0.00133
***
999,0
```

OUTPUT SUMMARY

ANIMAL PRODUCTION COST ($/KG OUTPUT)		PERFORMANCE MEASURES		
0.0841	SPACE	0.3976	PHYTO	SPACE COST ($/KG)
0.0000	HEAT	0.1527		RECIRC COST ($/KG)
0.0032	AERATION	0.1291		LABOR COST ($/KG)
0.0834	INTAKE WATER (PUMPED)	49099.2984	PHYTO	TANKS AREA (M2)
0.5472	RECIRCULATION PUMPING	1.0000	-"-	TANKS DEPTH (M)
0.0000	FEED:ARTIFICIAL	0.6530	-"-	TANKS FLOW(M3/SEC)
0.6794	:PHYTOPLANKTON	0.6904	-"-	CONVERSION EFFIC
0.0524	WASTE TREATMENT	10.0292	ANIMAL	INDIVID WEIGHT(G)
0.2462	LABOR,REGULAR	5000.0000	-"-	BATCH WEIGHT (KG)
0.1000	-"- ,SUPERVISORY,&C	5714.4768	-"-	NUMBER OF TRAYS
0.1262	LARVAE	0.3073	-"-	CONVERSION EFFIC
1.9220	TOTAL	189.0000	-"-	DAYS TO HARVEST
879.1415	TOTAL CAPITAL (000)	197.5353	ANN'L	TOTAL CAP (000)

ARTIFICIAL UPWELLING MARICULTURE: RECOMMENDED REVISIONS TO BE MADE TO THE AQUACULTURE BUDGET GENERATOR COMPUTER PROGRAM

by

DR. GEOFFREY P. ALLEN

March 26, 1977

Revisions to the aquaculture budgeting program 3/26/77

Introduction

This report consists of three sections.

I. Improvements to the program and notes for conversion to FØRTRAN. Several redundant statements have been deleted. Removal of the option to evaluate single batch or continuous production has permitted further simplification. (Single batch production can still be evaluated if the interval between batches is set equal to or greater than time to harvest.) Statements which made reference to a compartment size table, not used in this version of the program, have also been deleted.

II. Modifications to the program. Five sets of modifications are described and the required changes in the program noted.

III. Revised definitions of variables. The list of variables and their definitions which was provided in an earlier report is updated to reflect changes in parts I and II. In addition, sources are given for baseline values of variables listed in INDATA.

Future work

Some additional documentation may be sought for the baseline values of variables in the input data file. Part of the intent of the program layout is that the degree of confidence in the values assumed for the variables should be exposed. Concern here can lead in two directions:

(1) an investigation of the sensitivity of the calculated costs to changes in assumed values of variables where justification of those values is weak; and

(2) a search for more reliable or empirically validated values relating to the species and site under consideration.

It is important to remember that the cost of production estimates can only be fully validated when commercial, or large scale pilot plant operations occur. We are measuring changes in cost assuming that the model is correct. It would therefore seem appropriate to work through the cost components of greatest magnitude in detail to ensure that the best representation of the real-world situation is being modeled. An investigation of the impact of modifications to the existing technology can be carried out at the same time. These steps should provide firmer delineation of areas of ignorance concerning biological responses, feasible technologies and costs, and the importance of better information in reducing, or in giving more precision to, estimated cost of production.

I Improvements to Program and Notes for Conversion to FØRTRAN

Note 1: Some simplification can be achieved if the package is condensed to a main program (AQUA2B) and an input data file (INDATA). A working data file is still required but it need not be in binary form. The working data file should retain updated parameter values until such time as the original data set from INDATA is wanted. The easiest way to achieve this operation would be to define two data files INDATA and CURRDATA where CURRDATA fulfills the role that BNAR has at present. Then at line 54511 insert another branch statement to a set of statements which will

(i) read INDATA from a disk file

(ii) write the data onto CURRDATA

This option would be mandatory the first time the program was run, and would be selected whenever a return to the original data set was desired.

Note 2: FTN = FØRTRAN

Note 3: * indicates line(s) marked are redundant in present program and can be removed.

AQUA2B lines	comments
8000-11500	File declaration cards. Format will depend on system requirements. FILETYPE = 7 is a designation which permits disk file to be read regardless of its record size or blocking characteristics.
12500	If Note 1followed, the array SUMTIT can be read in as a data table.
15050	TEST is a TRUE/FALSE variable
16000-17500	Omit in FTN.
18000-30150	These would not be declaration in FTN and hence constitute part of the program.
*27280-27310	Omit. Only one table

lines	comments
*27340-27370	Omit. Only one table.
*30100-30150	Omit. Refers to compartment size table, not used.
*31000	Omit.
31500	Omit in FTN. Use assigned GØTØ statement on program.
34000-44300	These are data tables in FTN.
*44000	Omit.
*44400-44440	Omit. Debugging aid.
*45000-47000	Omit. Procedure refers to section no longer used.
47000	This is the last line of declaration statements. In FTN the format statements (lines 18000-30150) could be placed anywhere after this line.
*47100	Change to 47100 READ(DISKIN3, 901, A903[*]); A change in INDISK must also be made. See page I-4 . If Note 1 followed, read statement in DØ loop. Add error branch. See line 54511 below.
*47200-47700	Delete
49000	If Note 1 is followed, add error branch. See line 54511 below.
49100-49110	If Note 1 followed, omit.
*51500	Omit. Not used.
52000-54517	Can use assigned GØTØ in FTN provided default for incorrect value of IWANT transfers control to line 54527.
54511	If Note 1 followed insert branch statement here. At the statement branched to (i) Read from INDATA (ii) Write to CURRDATA (iii) Insert lines to calculate number surviving, that is ACP/INDISK lines 12134-12141(except line 12140 would then read 12140 FØR I:=1 STEP 1 UNTIL 901 DØ) (iv) Write values for number surviving on disk file (BNAR2). The error branches on lines 47100 and 49000 should transfer control to this group of statements. If either disk file of current data cannot be read (e.g. if it has been removed) then original values of parameters will be placed in these files.
*54530	Change to 54530 TABLES:

lines	comments
*54540-54550	Omit. Only one table.
*54610	Change to 54610 WRITE (DISKIN3, 901, A903 [*]);
*54620	Change to 54620 END;
*54630-54950	Omit. Only one table.
*54960	Change to 54960 GØ TØ PICKØNE;
*6000-84000	Omit. No longer used in this form.
91030	In FTN use assigned GØTØ statement.
*97510-97560	Replace by the following statements which provide for printing out sections of the mortality adjustment table.
97510 LIST 3:	WRITE (REMØUT, FMTØUT6);
97520	READ (REMIN,/,LØW, HIGH);
97530	IF LØW LEQ 0 THEN LØW:=1;
97540	IF HIGH GTR 900 THEN HIGH: = 900;
97550	BEGIN
97560	WRITE (REMØUT,< "MØRTALITY ADJ. FACTØR TABLE"/>):
97565	FØR I: = LØW STEP 5 UNTIL HIGH DØ
*97590-97650	Omit. Table no longer used.
99122-99220	Changes are described in section IIb.
*98310-102650	A number of changes are required in this section of the program to facilitate removal of the single batch option. They are described in detail below.
98310	Change to 98310 FØR I:=311, 432, 436, 452, 453, 454, 455, 456, 463, 513, 621, 915, 916, 924
99100	Change [513] to [514]
100020	Change [621] to [612]
100032	Change [432] to[435] and [621] to [612]
100039	Change [436] to [437] and [432] to [435]
100040	Change [463] to [462] and [621] to [612]

line	comments
100970	Change [915] to [925]
100935	Delete
100992	Change [915] to [925]
101400	Change to 101400 VAR[621]:= *+VAR[612];
101500	Change to 101500 VAR[513]:= *+VAR[514];
101600-101670	Change to 101600 VAR[432]: = *+ VAR[435]; 101650 VAR[436]: = *+ VAR[462]; 101670 VAR[463]: = *+ VAR[462];
101850	Change [925] to [915]
101900	Change [926] to [916]
101910-101920	Delete. The logic appears at fault here. Number of transfers should not be cummulated for continuous production. The output will need the same number of transfers regardless of whether it is single batch or continuous production.
102130	Delete.
102150	Change to 102150 VAR[900]:= VAR[924]/VAR[99];
102200-102650	Delete
*106300	Delete. not used.
*107000-107450	Delete. Alternative listing of capital cost is provided in the program.
212000	Change 30.7 to 0.012. Error made in translating units to metric. Influences capital cost of heating equipment. No change in results from present program.
*223500	Delete. Single batch option eliminated
*224000	Change to 224000 VAL: = VAR[921]* 365.0/VAL;
INDISK 8000	ARRAY A[0:13], A903[0:900]; Eliminates use of arrays A910, A911 not required. Lines 12130-12146 will then require the following changes.
*12130	Delete
*12140	Change 301 to 901

lines	comments
INDISK	
*12142-12146	Delete
*12150	WRITE (DISKØUT2, 901, A903,[*]);
*12160 12170	DELETE

II Modifications to program

a.) Addition of artificial nutrients and calculated compensation depth dependent on nutrient level.

Order of calculations

1. Nutrient concentration at inlet is sum of nutrient concentration in deep sea water and nutrients added artifically.

$$N_t = N_d + N_a$$

where

N_t is total nutrient concentration in inlet water G-N/CM^3.

N_d is nutrient concentration in deep sea water G-N/CM^3.

N_a is nutrient concentration added artifically G-N/CM^3.

2. Compensation depth is a function of nutrient concentration

$$D_k = a + \frac{b}{N_t} \cdot D_c(448 \times 10^{-9})$$

where

D_k is the calculated compensation depth (CM).

D_c is the standard compensation depth (CM).

$D_c = 400$ when $N_d = 448 \times 10^{-9}$ is assumed.

Comment: D_k has been expressed in this way to make use of information available in D_c. In the function the values a=0.0, b=1.0 have been used. The function has the properties that as $N_t \to 0$, $D_k \to \infty$, as $N_t \to \infty$, $D_k \to 0$, and when $N_t = N_d$, $D_k = D_c$.

Modifications to the program are described in section IIb.)

b.) Actual depth not compensation depth of phytoplankton tank used in calculation.

Order of calculations

1. Depth factor D´ is calculated according to the formula

$D' = D - D^2/2D_k$ where D´ is the apparent pool depth (cm)

D is the actual pool depth (cm)

2. Exposure factor, K

$K = \frac{\dot{V}}{D'}$ where $\dot{V}$ is the flow into the phytoplankton tank ($CM^3CM^{-2}sec^{-1}$)

$\frac{1}{K}$ is the apparent detention time (sec)

$\frac{1}{K}$ measures the apparent length of time taken for a volume of water to be totally replaced. It is less than the actual detention time e.g. if $D' = 200$ and $\dot{V} = 0.1$ then $\frac{1}{K} = 20000$ seconds or about 5½ hours. The actual detention time if D=400 would be 40000 seconds or about 11 hours.

3. Efficiency of conversion, η_1

$$\eta_1 = 1.0 + b_1 k = b_2 k^2$$

Note: a functional form which avoids the decreasing value of apparent depth that occurs at higher values of actual depth is

$D' = a(1-e^{-bD})$ where a and b are parameters

If a=200, b=0.01 the function gives similar values to the existing one.

e.g.

D	D′ using original function	D′ using suggested replacement
0	0	0
10	9.9	19.0
100	87.5	126.4
200	150.0	172.9
400	200.0	196.3
600	150.0	199.5
800	0	199.9

A better fit to the data could be found for this suggested non-linear function. The above values are solely for illustration.

Modifications to the program

AQUA2B

Lines 99122-99220 should appear as follows:

```
99122          VAR[60]:= VAR[644]+ VAR[20];
99124          VAR[61]:= 0.0 + 1.0*(VAR[20]* VAR[22]/VAR[60]);
99130          IF VAR[18]GTR VAR[61]THEN
```

```
99131 }
99133 }          unchanged

99135            2(x2, F8.1)>, VAR[18], VAR[61]

99137 }
99140 }          omit

99150            VAR[51]:=VAR[18]-(VAR[18]**2.0)/(2.0*VAR[61]);

99155            IF VAR[51]LEQ 0.0 THEN VAR[51]:=0.0001;

99160 }
99214 }          unchanged

99220            VAR[54]:=6.25* VAR[60];
```

Line 99155 is added since the functional form permits negative values of VAR[51], which would be meaningless and would interfere with subsequent calculations.

c.) Cost of artificial nutrients included in total cost.

Order of calculations

1. Cost of artificial nutrients

$$C_n = F \cdot R \cdot C_n \ (86.4) \ 365.0/Q$$

where

C_n is cost of nutrients $/kg output

F is flow rate into phytoplankton tanks cm^3/sec

R is rate of addition of nutrients g-N/cm^3

C_u is unit cost of nutrients $/kg-N

Q is output from the facility kg/year

2. Cost of feed (phytoplankton)

$$C_p = C_{ps} + C_{pr} + C_{pl} + C_n$$

where

C_p is cost of feed $/kg output

C_{ps} is cost of space for growing phytoplankton $/kg output

C_{pr} is cost of recirculation pumping in phytoplankton tanks $/kg output

Modifications to the program

AQUA2B
line

224500 VAR [930]:= VAR[663]*VAR[664]*VAR[610]*86.4*365.0 /VAL;

237000 Change to 237000 VAR[943]:=VAR[930]+VAR[932] +VAR[934]+VAR[936];

Comment: The cost of artifical nutrients does not appear separately in the printed output but can be calculated as the difference between the cost of feed (phytoplankton) and its major components (space, recirculation pumping and labor).

d.) Inclusion of manpower requirements.

Order of calculations

1. Cost of labor in animal production facility

$$C_L = C_a \cdot A/B$$

where C_L is the cost of labor in the animal production facility ($/day)

A is the number of animals started per batch

B is the interval between batches (days)

C_a is the share of cost of transfer and harvest

2. Total regular labor requirement

$$C_{TL} = (7C_L + C_{PL}/52)/W$$

where C_{TL} is the regular labor requirement for transfer, harvest and cleaning (hours/week)

C_{PL} is the total cost of cleaning phytoplankton tank ($/year)

W is the regular labor wage rate ($/hr)

3. Number of supervisory and technical staff

$$S_T = C_b \cdot V/S_a$$

where S_T is the total number of full time equivalent supervisory and technical staff required (#)

C_b is the overhead cost assigned to output ($/kg)

V is the output per year (kg/year)

S_a is the annual salary for supervisory and technical staff

Modifications to the program

AQUA2B
line

49100 Change 25 to 27

102130 (This line can be deleted in present program. Replace by VAR[706]:=VAR[706]+ VAR[900];
This treats harvest as another transfer operation, carried out at the same rate and with same labor.)

220125 Move line 224000 to this location

220300 VAR[707]:=VAR[706]* VAR[926]/VAR[806];

220600 VAR[957]:= (VAR[707]* 7.0+VAR[708]/52.0)/VAR[611];

220700 VAR[958]:= VAR[701]* VAL/VAR[809];

224000 Delete after moving to line 220125.

Change to 241000 VAR[947]:=VAR[707]*365.0/VAL;

252300 Change 955 to 957

INDATA

11800 809 16.000.0 ANNUAL SALARY, TECH & SUPER LABØR ($)
(these must line up correctly for format I3, F20.2,9A6)

SUMDATA

2700 REG LABØR (HRS/WK)

2800 SUPER & TECH LABØR (#)
(these must line up correctly for format 4A6)

e.) Changes in baseline assumptions

1. Var[27], coefficient b_2 in phytoplankton conversion efficiency function

   Source Memo of 2/25/77 LVH. Original value did not include light attenuation at depth.

2. Var [24], unit flowrate into phytoplankton tanks

   Source Memo of 2/25/77 LVH. Corresponds to turnover rate of 1.15 times per day in 100cm deep pool rather than 1.5 times per day assumed previously.

Modifications to the program

INDATA

line 1700 24 0.001331 FLØWRATE TØ PHYTØ TANK (CM3/CM2/SEC)

1900 27 -134000000.0 CØEFF B2 IN PHYTØ EFFIC FN (#)

(these must line up correctly for format I3,F20.2, 9A6)

III. Definitions of Variables and Sources for Assumed Values

Notes: key to abbreviations in sources.

EPA report	Blecker, H.C. and T.M. Nichols. Capital and Operating Costs of Pollution Control Equipment Modules, Vol II- Data Manual. Environmental Protection Agency Report EPA R5-73-0236 July 1973.
Gravitz	Gravitz, N., L. Gleye, G. Tchobanoglous and R. Shleser "Preliminary Acute Toxicity Studies of Some Inorganic Compounds in Homarus americanus" unpublished manuscript.
Lightburn & Roels	Lightburn K.D. and O.A. Roels "Economic Analysis of a Mariculture Plant" unpublished manuscript, December 1971.
Liao and Mayo	Liao, P.B. and R.D. Mayo "Salmonid Hatchery Water Reuse Systems" Aquaculture 1:317-35. 1972.
LVH	Estimate by Mr. L. van Hemelryck.
T/S	Tchobanoglous, G. and R. Shleser. "Waste Treatment Costs for Saltwater Aquaculture Facilities" unpublished manuscript. [may have been published in Aquaculture.]

III. Definitions of Variables (Revised 3/26/77)

Variable Number	Variable Value	Description	Units
1	0.0	Not operational	
2	900.0	Time limit on growth. Purpose to permit examination of production cost over selected intervals. Overridden if either maximum harvest weight [920] or maximum number of transfer cycles [10] is reached. Source Limit on array storage of 900 in this program.	day
4	0.0	Ammonia concentration in intake water Source None. Zero value assumed.	mg-N/l
5	0.9	Efficiency of oxygenation in animal tank recirculation pumping unit Source Assumption.	propn.
8	1.0	Number of recirculation pumps in animal tanks. Source Assumption: Increasing the number of pumps will increase capital costs but permits isolation of each section of the production unit, a protection against epidemic losses.	(#)
10	5.0	Number of transfer cycles in the animal system. In conjunction with [11] it will determine harvest day, which will occur when a transfer is required but none is available. Overridden if maximum harvest weight [920] or maximum growth time [2] is reached. Source Assumption.	#
11	4.0	Ratio of final weight per tray to initial weight per tray. When exceeded one tray is transferred to several others, the number being the value in [11]. Source Assumption. In combination with variable 19 it determines the limits in which the load of a tray will lie.	#
13	0.0	Cost of deep sea water delivered to site excluding in-site pumping costs. Source No cost figure available. Will depend on whether water supplied as by-product from industrial process or pumped specifically for aquaculture facility.	$/m^3
14	2.65	Ratio of total animal weight to wet meat weight Source Experimental observation-LVH	g total /g wet meat

Variable Number	Variable Value	Description	Units
15	2.50	Ratio of total meat weight to meat protein weight (dry matter basis) Source Experimental observations-LVH.	g dry meat /g dry prot.
16	0.0	Reserve phytoplankton tank capacity as proportion of utilized capacity. Source Assumption. No reserve capacity to protect against accidental loss of the use of a tank.	propn.
17	5.0	Ratio of clam wet meat weight to dry meat weight Source Experimental observation-LVH.	g total /g dry
18	100.0	Depth of phytoplankton tanks Source Depth of experimental pools, St. Croix-LVH.	cm
19	20.0	Maximum weight of animals in a tray. Source Design criterion, greatest weight which can be conveniently manipulated without mechanical aids-LVH.	kg
20	0.000000448	Concentration of nutrients in deep sea water Source Experimental observation, St. Croix-LVH.	$g\text{-}N/cm^3$
22	400	Specific compensation depth for phytoplankton production Source Assumed value for seawater with 448×10^{-9} $g\text{-}N/cm^3$ of nutrients-LVH.	cm
24	0.001331	Unit flowrate into phytoplankton tanks Source Calculated value based on a turnover rate of 1.15 times per day in tanks of 100 cm depth-LVH.	$cm^3/cm^2/sec$
26	0.0	Coefficient b, in phytoplankton conversion efficiency function Source see variable 27.	sec
27	-1340000000.0	Coefficient b_2 in phytoplankton conversion efficiency function Source Based on calculations by LVH. Uses experimental observation that efficiency of conversion is 0.69 when flowrate into 100 cm deep tank is 0.001331 $cm^3/cm^2/sec$ and efficiency is zero when flowrate is 0.002778 $cm^3/cm^2/sec$. See memos 5/26/76 and 2/25/77.	sec^2
29	3.0	Animal feed rate decision ratio of actual feeding rate to biologically efficient (maximum conversion rate) feeding rate for animal Source Assumption recognizing the trade-off between biologically efficient production of an animal and the cost of providing it with a suitable environment by LVH.	#

Variable Number	Variable Value	Description	Units
31	0.000000011808	Biologically efficient specific feed supply rate Source Calculation based on experimental observation-LVH.	g-prot/sec /lg animal
34	-16.89145	Coefficient a_0 in animal conversion efficiency function	#
35	-1.891173	Coefficient a_1 in animal conversion efficiency function	#
36	-0.0518	Coefficient a_2 in animal conversion efficiency function Source Animal conversion efficiency function fitted to limited experimental and theoretically required values-LVH.	#
47	0.001	Cost of larva on intering the production system Source Assumption. Reflects unknown hatchery costs. No known market exists from which values could be obtained.	$/head
51		Phytoplankton tank apparant depth	cm
52		Phytoplankton tank exposure factor (its reciprocal is the exposure time)	/sec
53		Phytoplankton conversion efficiency	g out/g in (Nitrogen or protein basis)
54		Equivalent protein concentration (nitrogen x 6.25) in deep sea water	g-prot/cm^3
55		Rate of protein production in phytoplankton tanks	g-prot/cm^2/sec
56		Actual specific feeding rate of animals	g-prot/sec/1 g animal
57		Specific animal conversion efficiency	g-prot out/g-prot in/1 g animal
58		Coefficient to convert growth measured as gain in protein to growth measured as total wet weight	g-total/g-prot
59		Rate of feeding an individual animal based on the average weight in a batch on that day	g-prot/sec/ animal

Variable Number	Variable Value	Description	Units
60		Nutrient concentration in intake water to phytoplankton tanks	$g\text{-}N/cm^3$
61		Calculated compensation depth, dependent on nutrient concentration	cm
98		Weight of individual animal, adjusted for mortality, at which a transfer to more trays must be made	g/animal in
99		Number of animals started on one tray at the start of the growth calculations	$ animals in/tray
101	10000.0	Cost of land Source Highly location specific. Reflects a premium of about 100% over prime agricultural land prices in the United States.	$/ha
102	3000.0	Area of facility utilizable by building or tank structures Source Assumed that four-fifths of the land area can be actively used and one-fifth will be waste, access roads, etc.	m^2/ha
103	10.0	Capital cost of phytoplankton tanks excluding excavation Source Lightburn and Roels (1971) provide calculations which indicate a capital cost for phytoplankton tanks 12 feet deep excluding excavation costs of between $13.50 and $15.00 per square meter. Approximately three-fourths of the cost is for piping and ancillary equipment. If this part of the cost is cut to one-third for a one meter tank but costs of lining remains the same then capital costs range between $7.10 and $8.15 in 1971 dollars. The baseline figure is therefore a high cost, which might be reduced by less substantial engineering standards.	$/m^2$
104	5.0	Life of phytoplankton tanks Source Assumption. This value is lower than the lifetimes typically applied to equivalent structures but is intended to recognize the likelihood of obsolescence in an activity where the technology is relatively undeveloped. The class life system (ADR) used by the U.S. Treasury has the following asset depreciation ranges (in years)	year

Variable Number	Variable Value	Description	Units
		Lower Limit / Guideline Period / Upper Limit	
		Agricultural machinery & equipment: 8 / 10 / 12	
		Farm buildings: 20 / 25 / 30	
		Light trucks: 3 / 4 / 5	
105	0.0	Cost of cover Source Assumed that no shading or other protection would be required for animal tanks	$/m^2
106	5.0	Life of cover Source See variable 104.	year
107	0.0063	Ratio of office laboratory and storage space area to phytoplankton tank area Source Assumption based on providing approximately 250m^2 of office, laboratory and storage area for an operation using 40,000m^2 of phytoplankton tank and producing 1 metric ton per day.	#
108	20.0	Capital cost of animal tanks, complete Source Estimate by LVH.	$/tray
109	5.0	Life of animal tanks and equipment Source See variable 104.	year
110	3.0	Stacking factor (Number of trays per square meter of structure) Source Assumption.	#
111		Effective capital cost of utilized land	$/m^2
112		Rental cost of raw land	$/m^2/yr
113		Annual cost of phytoplankton tanks (excluding excavation)	$/m^2/yr
115		Annual cost of cover for phytoplankton or animal tanks	$/m^2/yr
116		Capital cost of office and storage space expressed per area of tank	$/m^2 - tank

Variable Number	Variable Value	Description	Units
117		Annual cost of office and storage space	$/m^2 - tank/yr
118		Annual cost of animal tanks, complete	$/tray/year
119		Total capital cost of phytoplankton tanks	$/m^2
120		Total annual cost of phytoplankton tanks	$/m^2/yr
121		Total capital cost of animal tanks	$/tray
122		Total annual cost of animal tanks	$/tray/yr
123		Total cost of excavating phytoplankton tanks	$/m^2
124		Length of embankments, phytoplankton tanks	m
125		Cross-sectional area of phytoplankton tank embankments	m^2
126		Total cost of excavation work, phytoplankton tanks	$/m^2 tank
127		Annualized cost of excavation work, phytoplankton tanks	$/m^2/year
200		Heat input required to maintain operating temperature	Kcal/l
201	20.0	Mean ambient sea-water temperature as it enters the system Source Approximates average value observed in phytoplankton tanks, St. Croix.	^oC
202	0.0	Operating temperature of tanks Source Assumes no external temperature control.	^oC
203	0.5	Efficiency of heat exchanger, proportion of available heat recovered.	#
204	0.0	Not operational	
205	10.0	Life of heat exchanger Source see variable 104.	year
206	2.0	Annual maintenance cost for heat exchanger Source EPA report.	% of capital cost/year

Variable Number	Variable Value	Description	Units
207	0.0	Not operational	
208	10.0	Life of conventional oil or gas fired boiler Source Assumption.	years
209	3.0	Annual maintenance cost, excluding regular labor, conventional fired boilers Source EPA report.	% of capital cost/year
210	4.0	Labor requirement for conventional fired boiler Source EPA report.	hrs/day
211	8.0	Maintenance engineer wage rate Source typical skilled labor rate, United States.	$/hr
212	0.9	Efficiency of conventional fired boiler Source Assumption	proportion
213	11.00	Cost of fuel oil Source Assumes 34500 kcal/gal and 38 cents/gal for #2 fuel oil	$/mil Kcal
214	0.0	Loss of heat within system Source Assumed zero since no external heating employed.	^{o}C per pass
215		Heat input required to maintain system at operating temperature	Kcal/day
216	0.0	Area of heat exchanger Source Assumed zero. No external heat source employed.	m^2
217		Heat input provided by recovery through heat exchanger	Kcal/day
218		Standard capital cost, heat exchanger	$
219		Capital cost of heat exchanger, adjusted for changes in cost of construction index	$
220		Annual cost of heat exchanger, including capital recovery charge and maintenance cost	$/year
221		Heat input required from conventional source	Kcal/day
222	50.0	Reserve capacity provided for conventional heater Source Assumption. Value will depend on extent to which animals can tolerate acute temperature drop to ambient water temperature.	% of utilized capacity

Variable Number	Variable Value	Description	Units
223		Total conventional heater capacity required	Kcal/hr
224		Standard capital cost of conventional heater	$
225		Capital cost of conventional heater, adjusted for changes in cost of construction index	$
226		Annualized capital cost for conventional heater	$/year
227		Annual operating cost for conventional heater	$/year
228		Total annual cost for conventional heater	$/year
229		Operating temperature in animal tanks. If [202] greater than zero; [229]=[202], otherwise [229]=[201], ambient temperature	^{o}C
301	0.85	Efficiency of pump Source EPA report	propn
302	3.0	Pump lift-intake Source Assumes water is delivered to site and represents head loss within the plant only.	m
303	100.0	Intake pump standby capacity Source Assumes single intake pump and need for continuous operation.	% of utilized capacity
304	10.0	Life of pump Source see variable 104.	year
305	3.00	Pump annual maintenance cost Source EPA report.	% of capital cost
306		Animal tank recirculation pumping power total requirements	Kw
307		Capital cost of individual pump (successively: phytoplankton recirculation, animal recirculation)	$
308		Annual cost of pump equipment (successively, same order as [307]).	$/yr
309		Operating cost of pumps (successively, same order as [307]).	$/yr
310		Total cost of recirculation pumping in animal tanks	$/yr

Variable Number	Variable Value	Description	Units
311		Total cost of intake pumping	$/yr
312	0.00000025	Animal tank recirculation pumping power coefficient Source LVH. Function assumes that resistance of shellfish to water passage increases with their depth in tray, represented by the square of grams of animal per tray. Equivalent to assumption that to drive 2.5 l/sec of water through a tray containing the maximum of 20 kg of shellfish requires 0.1 kw of pump power	watts/(g of animals in tray)^2
320		Total cost of intake pumping into phytoplankton tank	$/yr
321		Total cost of intake pumping direct to animal tanks	$/yr
322		Total cost of recirculation pumping in animal tanks	$/yr
323		Total cost of recirculation pumping in phytoplankton tanks	$/yr
326		Pumping capacity, intake to phytoplankton tanks	Kw
327		Pumping capacity, intake direct to animal tanks	Kw
328		Pump capacity required for each isolated animal tank recirculation circuit	Kw
329		Pump capacity required for recirculation in phytoplankton tanks	Kw
330		Total capital cost of pumping equipment (Sum of steps described under [307]).	$
331		Annual cost of pumping equipment (Sum of steps described under [308]).	$/year
401	2400.0	Cost of construction index for inflating those cost functions constructed on the basis ENRCC=2000 Source Engineering News Record.	#
402	1.0	Screening	
402	1.0	Filtration	

Variable Number	Variable Value	Description	Units
404	0.0	Nitrification	
405	0.0	Carbon absorption	
406	0.0	Ozonation	
407-410	0.0	Not operational	

The variables [402] to [406] are used for both capital and operating costs. They take the value 1 if the process is included and 0 if the process in not included. It is assumed that if the equipment is installed it will be used, hence variables [407] to [410] which formerly acted to include operating and maintenance costs, when desired, no longer serve any purpose. The functions used are described at the end of this section.

411	5.0	Ozone dosage required for sterilization Source T/S	mg/l
412	22.0	Electricity consumption in ozone production Source T/S	kwh/kg
413	0.04	Electrical power cost Source Typical cost for medium size users, United States.	$/kwh
414	0.9	Proportion of flow in animal tanks recirculated Source Assumption.	propn.
415	0.1	Proportion of recirculated water treated with carbon absorption Source T/S. Only operates if var[405]=1.	propn.
416	0.0	User specified flowrate through animal tanks. If set to zero then var [414] determines flowrate.	cm^3/sec/ g animal
417	90.0	Efficiency of waste treatment system in removing suspended solids Source Assumption. Not supported by data.	%
418	90.0	Efficiency of waste treatment system in removing nitrates Source Assumption. Not supported by data.	%
419	0.12	Environmental Protection Agency proposed discharge guideline Source T/S	g suspended solids/g feed
420	0.8	Rate of solid waste production Source T/S. Based on experimental observation on lobster.	g suspended solids/g food fed, dry matter basis

Variable Number	Variable Value	Description	Units
421		Rate of removal of suspended solids in single pass through discharge waste treatment	g-ss/l
422		Rate of flow in recirculation circuits in animal tanks	l/day
423		Required flowrate through discharge waste treatment plant to meet Environmental Protection Agency regulations	l/day
424		Total capital costs of waste treatment plant based on ENRCC index of 2000	$
425		Current capital cost of waste treatment plant based on current ENRCC index	$
426	10.0	Life of waste treatment plant Source See variable 104.	year
427		Annual cost of waste treatment plant	$/yr
428		Capital cost of waste treatment plant per 1000 liters treated	$/Kl
429		Operating cost of waste treatment plant per 1000 liters treated	$/Kl
430		Total cost of waste treatment per 1000 liters treated	$/Kl
431	0.003	Alternative form of Environmental Protection Agency proposed discharge guidelines Source T/S.	g suspended solids/g animal weight/day
432		Cumulated rate of metabolite production, theoretical maximum, continuous batch	g-N sec/ animal in
	(179300)	Suspended solids discharge allowance under Alternative Environmental Protection Agency regulations (see [431])	g-ss/day
	(179800)	Weight of suspended solids actually produced	g-ss/day
433		Suspended solids discharge allowance under Environmental Protection Agency regulations (see [419])	g-ss/day
	(179400)	The least limiting discharge allowance	g-ss/day

Variable Number	Variable Value	Description	Units
435		Rate of metabolite production, theoretical maximum based on conversion efficiency and expressed as nitrogen, mortality adjusted.	g-N/sec/animal in
436		Cumulated rate of un-ionized ammonia production in animal tanks	g-N/sec/animal in
437		Rate of un-ionized ammonia production in animal tanks (based on dissociation proportion of total theoretical ammonia production)	g-N/sec/animal in
438	(102950)	Calculated flowrate required in animal tanks to meet safe metabolite loads	cm^3/sec/g
	(103040)	Actual flowrate in animal tanks	l/day
439		Rate of deficit in oxygen supply. If less than zero, excess oxygen supplied, no additional aeration. If greater than zero, additional aeration supplied.	mg/hr
440		Maximum ammonia concentration allowed in animal tanks	mg/l
441	10.0	Design ammonia concentration (% of concentration causing 50% mortality in 96 hours-96 hours LC_{50}) Source Assumed suitable safety margin.	%
442	1.2	Acute toxic ammonia concentration Source Gravitz et al. Refers to observations with 1g and 3g lobsters. No data for clams.	mg/l
443	0.0	Rate of flow of (surface) seawater direct to animal tanks Source Assumed zero under present system.	cm^3/sec/g animal
444		Weight of feed entering the animal tanks (dry matter basis)	g/day
445	8.0	Operating pH Source Assumed same as seawater (7.8-8.1)	#
452		Capital cost of screening equipment in waste treatment plant	$
453		Capital cost of mechanical filtration equipment in waste treatment plant	$
457		Operating cost of screening equipment in waste treatment plant	$/day

Variable Number	Variable Value	Description	Units
458		Operating cost of mechanical filtration equipment in waste treatment plant	$/day
462		Rate of production of suspended solids in animal tanks, adjusted for mortality	g-ss/sec/ animal in
463		Cumulated rate of production of suspended solids in animal tanks, continuous production	g-ss/sec/ animal in
493		Rate of flow of water from phytoplankton tanks and direct from sea into animal tanks	cm^3/sec/g
494		Proportion of animal tank flow recirculated. If [416] is equal to zero, [494] equals [414]. If insufficient flow to maintain safe metabolite load, warning message is issued.	#
501	0.88	"Metabolic body size" factor in oxygen consumption equation Source Unpublished experiments on lobster (Schuur) No data for clams.	#
502	5.0	Minimum tolerable oxygen concentration in animal tanks Source Assumed value for lobster. Adult animals would tolerate much greater acute deficits (below 3mg/ℓ). No data for clams.	mg/ℓ
503	-0.013908	Coefficient b_o in specific oxygen consumption function Source Calculated from unpublished experimental data on lobster by Schuur. No data for clams.	mg/hr /lg animal
504	0.6	Aeration efficiency; surface aerator Source Based on Liao & Mayo, figure 5, average oxygen deficit of 2 mg/ℓ.	kgO_2/kwh
505	1.0	Not operational. Was used as conversion factor for kwh to HP including efficiency loss.	
506	500.0	Capital cost of aerator Source Subjective estimate by Tchobanoglous.	$/installed Kw
507	1.0	Maintenance cost of aerator Source EPA report.	% of capital cost/year
509	5.0	Life of aerator Source See variable 104.	year

Variable Number	Variable Value	Description	Units
513		Oxygen consumption rate, continuous production, adjusted for mortality	mg/hr/ animal in
514		Oxygen consumption rate in animal tanks, adjusted for mortality	mg/hr/ animal in
515		Saturation oxygen concentration in intake water (103500). Saturation oxygen concentration in recirculation water	mg/hr
517		Rate of oxygen consumption in animal tanks	mg/hr
519		Direct aeration capacity required	Kg/hr
520		Temperature corrected specific oxygen consumption rate	mg/hr/lg animal
601	0.0025	Phytoplankton tank recirculation pumping power requirement Source Extrapolation of existing recirculation pumping capacity in phytoplankton tanks at St. Croix.	kw/m^3
602	0.0	Cost of artificial feed Source Zero value indicates no artificial food fed.	$/kg
603	2.5	Composition of phytoplankton Source Experimental observations-LVH.	g-dry matter /g protein
604	0.0	Reserve capacity of feeding equipment Source Zero indicates none used.	proportion of utilized capacity
605	0.0	Frequency of feeding artificial food Source Zero indicates none used.	#/day
606	0.0	Capacity of feeding equipment Source None used.	m^2/day/unit
608	0.0	Life of feeding equipment Source None used.	year
610	0.0	Cost of added nutrients Source Not investigated, none used.	$/kg-N
611	4.0	Wage rate, regular labor Source Assumption. A high value for U.S. conditions. U.S. average wage for hourly paid workers in agriculture, Dec. 1976 was $2.84 per hour (USDA-Economic Research Service-Farm Labor) Fringe benefits typically at 10% to employer's cost.	$/hour

Variable Number	Variable Value	Description	Units
612		Rate of feeding animals of given weight, adjusted for mortality	g-prot/sec /animal in
618	0.0	Capital cost of feeding equipment Source None used.	$/unit
620	0.002413	Coefficient b_1 in temperature corrected specific oxygen consumption function Source See variable 503.	mg/hr/^{o}C /lg animal
621		Rate of feeding animals, continuous production, adjusted for mortality	g-prot/sec /animal in
640		Rate of feeding phytoplankton to animals, total plant	g-prot/sec
641	200.0	Capital cost of office, laboratory and storage space Source	$/m^2
642	15.0	Life of office, laboratory and storage space Source See variable 104.	year
643	0.0	Efficiency of animal tank recirculation loop waste treatment system in removing ammonia Source None used.	%
644	0.0	Rate of addition of artificial nutrients to phytoplankton tank intake water (Note: where these nutrients are added in dilute form e.g. sewage sludge, the volume is the total volume of deep sea water and diluted nutrient and the quantity is related to this volume) Source Assumes no addition.	g-N/cm^3
661		Utilized area of phytoplankton tanks in the plant	cm^2
662		Total area of phytoplankton tanks in the plant, including reserve	cm^2
663		Total flowrate of deep sea water into phyto-plankton tanks	cm^3/sec
664		Utilized volume of phytoplankton tanks	m^3

Variable Number	Variable Value	Description	Units
701	0.1	Cost of supervisory and technical labor Source Assumption. No data available.	$/kg output
703	20.0	Rate of transfer and harvest of animal trays (Represents number transferred into) Source Assumption.	trays/hour
704	1.0	Frequency of hand cleaning of phytoplankton tanks Source Assumption. Supported by typical cleaning frequency at St. Croix.	#/month
705	50.0	Rate of hand cleaning phytoplankton tanks Source Based on subjective estimate, LVH.	m^2/hour
706	(101002)	Number of transfers per tray at end of growth calculations	# transfers /tray in
	(102140)	Share of number of transfers per animal at end of growth calculations	# transfers /animal in
	(220240)	Cost of transfer and harvest	$/animal in
707		Total cost of labor associated with animal production facility	$/day
708		Cost of hand cleaning phytoplankton tanks	$/year
801	7.2	"Biological zero" temperature Source Not used in current program. Estimated for lobster from experimental observations. Apparent origin of temperature dependent growth function.	^oC
802	0.67	Metabolic body size coefficient used to determine feeding rate of animals Source Not used in current program. Estimated for lobster from experimental observations.	
803	3.0	Number of phytoplankton tanks. The reserve capacity of phytoplankton tanks, [16] should be made consistent with [803] which includes reserve area Source Assumption.	#
804	1.0	Unit cost of excavation work, phytoplankton tanks Source Lightburn and Roels (1971).	$/$m^3$ of embankment
805	0.01025	Initial weight of animals entering the system Source Experimental observations-LVH.	g

Variable Number	Variable Value	Description	Units
806	5.0	Interval between batches in continuous production Source Assumption.	day
807		Daily rate of weight gain for an individual animal for the given average weight	g/day/animal
808	0.8	Proportion of animals surviving after 180 days Source Subjective estimate-LVH.	propn.
809		Annual salary for supervisory and technical labor Source	$/year
900	(101000)	Number of trays in use per tray at start	#trays/tray in
	(102150)	Share of cumulated number of trays per animal started	#trays/animal in
	(106200)	Total number of animal trays in plant	#
901	1.0	Proportion of animals surviving at start of initial growth period. (Normally equal to one, but can be set to lower values in program is to be started from middle of a growth sequence, as when transferring from one system to another	animals surviving / animals started
915		Cumulated weight of animals in system, adjusted for mortality	g/animal in
916		Number of animals in the system, continuous production	#/animal in
920	10.0	Maximum weight of individual animal at harvest. Overridden if either maximum time [2] or maximum number of transfers [10] reached. Source Estimated average meat weight of marketed wild clams.	g
921	5000.0	Target output from a batch Source Assumption	kg/batch
922		Number of days to harvest of animals	day
924		Cumulated number of trays in use per tray at start of growth	#trays/tray in
925	(100970)	Weight of individual animal, at end of ith day, adjusted for mortality	g
	(102705)	Weight of animals in the system	g

Variable Number	Variable Value	Description	Units
927	0.1	Interest rate on sinking fund Source Represents a rate of borrowing for high risk ventures. Current rate on low grade corporate bonds ranges between 9½% and 11½%.	proportion
928	0.1	Return on capital, a rate charged the fixed investments of the firm Source See variable [927]. May be set at higher levels to calculate market price sought in high risk situation.	proportion
930		Cost of artifical nutrients added to phyto-plankton tanks	$/kg output
931		Cost of space for animals including land, buildings, tanks, trays	$/kg output
932		Cost of space for phytoplankton production including land and tanks	$/kg output
933		Cost of heating	$/kg output
934		Cost of recirculation pumping in phyto-plankton tanks	$/kg output
935		Cost of aeration in animal tanks	$/kg output
936		Cost of labor to maintain phytoplankton production	$/kg output
937		Cost of pumping intake water	$/kg output
938		Total area of phytoplankton tanks	m^2
939		Cost of recirculation pumping in animal tanks	$/kg output
940		Depth of phytoplankton tanks	m
941		Cost of feed supplement fed directly to animals	$/kg output
942		Flowrate into phytoplankton tanks	m^3/sec
943		Cost of feed (phytoplankton). This is the sum of [932], [934] and [936] plus the cost of artifical nutrients, if any.	$/kg output
944		Phytoplankton. Conversion efficiency	g out/g in (Nitrogen or protein basis)

Variable Number	Variable Value	Description	Units
945		Cost of waste treatment	$/kg output
946		Weight of individual animal at harvest	g
947		Cost of regular labor employed in animal production section	$/kg output
948		Weight of a single batch of animals at harvest	kg
949		Cost of supervisory labor	$/kg output
950		Number of trays of animals in the system with continuous production	#
951		Cost of larvae	$/kg output
952		Specific animal conversion efficiency	g protein out /g protein in /lg animal
953		Total cost of production	$/kg output
954		Number of days to harvest	#
955		Total capital cost	thousand $
956		Annualized capital cost	thousand $/year
957		Amount of regular labor for cleaning tanks and transferring animals	hrs/week
958		Supervisory and technical staff required	# full time equivalent

The following cost functions are fixed within the program:

Heat exchanger capital cost — $

$C = 5900\ Q^{0.5}$ where Q is the exchanger area — m^2

Source EPA report, converted to metric units

Conventional boiler capital cost — $

$C = 0.012\ Q^{0.548}$ where Q is heat output required — kcal/hr

Source EPA report, converted to metric units

Pump capital cost — $

$C = 560\ Q^{0.6}$ where Q is pumping power — kw

Source EPA report

Waste Treatment-screening, capital cost — $

$C = 18.243\ Q^{0.565}$ where Q is flowrate — ℓ/day

Source T/S

Waste Treatment-filtration, capital cost — $

$C = 31.758\ Q^{0.554}$ where Q is flowrate — ℓ/day

Source T/S

Waste Treatment-screening, operating and maintenance cost — $/day

$C = 181.063\ Q^{-0.169}$ where Q is flowrate — ℓ/day

Source T/S

Waste Treatment-filtration, operation and maintenance cost — $/day

$C = 16237.7\ Q^{-.346}$ where Q is flowrate — ℓ/day

Source T/S

The waste treatment functions apply to flowrates exceeding 50,000 ℓ/day.